CENTER FOR DRUG EVALUATION AND RESEARCH

Application Number 21-098

PHARMACOLOGY REVIEW(S)

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REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA:

KEY WORDS: Drospirenone, ethinyl estradiol

Reviewer Name: Krishan L. Raheja

Division Name: DRUDP

HFD#: 580

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Serial number/date/type of submission: Original submission/ 5-14-1999/NDA

Information to sponsor: Yes ( ) No ( )

Sponsor (or agent): Berlex Laboratories, Inc. Montville, New Jersey

Manufacturer for drug substance: Schering GmbH and Co, Germany

Drug name: <u>Progestin</u> <u>Estrogen</u>

<u>Code Name:</u> ZK 30595 ZK 4944
<u>Generic Name:</u> Drospirenone (DRSP) Ethinyl estradiol (EE)
<u>Trade Name:</u> Yasmin (drospirenone 3 mg and ethinyl estradiol 0.030 mg) tablets

<u>Chemical Name:</u> DRSP= (6B, 7B; 15B, 16B-Dimethylene-3-oxo-17a-pregn-4-ene-21-, 17-carbolactone (IUPAC)

EE =(1) 19-Norpregna-1,3,5(10)-trien-20-yne-3,17-diol,(17a)
(2) 19-Nor-17a-pregna-1,3,5(10)-trien-20-yne-3,17-diol

CAS Registry Number:

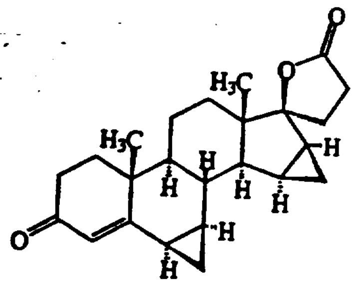
Drospirenone 67392-87-4
Ethinyl estradiol 57-63-6

Molecular Formula/ Molecular Weight:

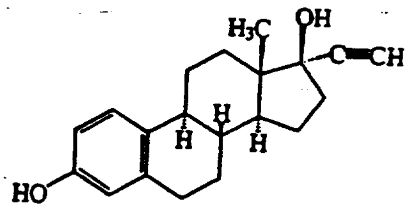
Drospirenone : $C_{24}H_{30}O_3$/366.5
Ethinyl estradiol: $C_{20}H_{24}O_2$/296.4

Structure:

Drospirenone



Ethinyl estradiol



Relevant INDs/NDAs/DMFs·

Drug Class: Steroid hormones.

Indication: Oral contraception

Clinical formulation: Tablet (3 mg Drospirenone and 0.030 mg ethinyl estradiol as active ingredients)

The inactive ingredients in Yasmin 21 tablets are: Lactose monohydrate NF, Maize starch NF, Modified starch NF, Povidone 25000 USP, Magnesium stearate NF, Hydroxypropyl methyl cellulose USP, Marcogol 6000 NF, Talc USP, Titanium dioxide USP, Ferric oxide pigment, yellow NF. Yasmin 28 tablets have 7 inert tablets and have the same inactive ingredients.

Route of administration: Oral

Proposed clinical protocol or Use: Drospirenone (DRSP) 3 mg and Ethinyl estradiol (EE) 0.030 mg will be used for oral contraception.

Previous clinical experience: Drospirenone (DRSP) and Ethinyl estradiol (EE) 0.030 mg has not been marketed anywhere in the world.

Disclaimer – use of sponsor's material: Some data are directly taken from sponsor's submission

Introduction and drug history: Yasmin 21 and 28 tablets (monophasic preparations) like all oral contraceptives are intended to prevent pregnancy. Its active progestational ingredient, drospirenone has not been marketed previously. Yasmin like other oral combination contraceptives act by suppression of gonadotropins.

Sponsor has claimed that drospirenone is a novel progestin with antimineralocorticoid activity and in combination with ethinyl estradiol it displays a favorable effect on the serum lipid profile with an increase in HDL and a decrease in LDL. It is devoid of any significant androgenic, estrogenic, glucocorticoid, and antiglucocorticoid activity. With these characteristics in combination with the antimineralocorticoid and antiandrogenic properties, the sponsor has claimed that drospirenone's biochemical and pharmacological profile closely resembles the natural hormone, progesterone inspite of the fact that the antimineralocorticoid activity of drospirenone is several times stronger than that of progesterone. Sponsor described DRSP "as the prototype of a new class of orally active progestogens with progesterone-like effects on salt and water excretion".

Studies reviewed within this submission: Studies include nonclinical pharmacology; nonclinical absorption, distribution, excretion and metabolism. These studies were not reviewed in detail in the original IND submission dated 10-7-1996. The mouse carcinogenicity study, which was ongoing when the original IND was submitted, is also now reviewed.

Studies <u>not</u> reviewed within this submission: The following toxicity studies were reviewed in detail in the original IND submission dated 10-7-1996. A copy of the original review dated 10-17-1997 is appended.

- Acute toxicity studies in female mice and rats

<u>Repeat dose toxicity studies:</u>

- 14-week dose range-finding study in female mice
- 14-week dose range-finding study in female rats
- 27-week toxicity study in female rats
- Carcinogenicity study in rats
- 27-week toxicity study in female monkeys
- 54-week toxicity study in female monkeys

<u>Reproductive toxicity studies:</u>

- Fertility study in female rats
- Teratology study in rats
- Teratology study in rabbits
- Embryotoxicity and teratology study in monkeys
- Peri and postnatal study in rats

<u>Mutagenicity studies:</u>

- Ames test
- HGPRT-test with V79 cells
- Clastogenic potential in the human lymphocyte test
- Unscheduled DNA synthesis

- Mouse micronucleus test
- DNA adduct analysis

PHARMACOLOGY:

Mechanism of Action: Combined oral contraceptives (COCs) act by suppression of gonadotropins. Although the primary mechanism of action is inhibition of ovulation, other alterations include changes in the cervical mucus (which increase the difficulty of sperm entry into the uterus) and in the endometrium (which reduces the likelihood of implantation).

Drug Activity Related to Proposed Indication: The inhibition of ovulation by drospirenone administered subcutaneously was tested in mice **(study No. 3996)** and in rats **(study No.4549).** The results were as follows:

Table 1. **Antiovulatory activity expressed as % animals/group with inhibited ovulation**

Dose (mg/animal/day)

Mice					Rats			
Vehicle	0.03	0.1	0.3	1.0	0.3	1.0	3.0	10.0
0	10	40	90	100	17	83	100	100

<u>Antiovulatory activity of Dropirenone by oral route **(Study No. 9963)**:</u> When rats were administered DSRP at dose levels of 0.1, 0.3, 1 and 3 mg/animal/day orally, the antiovulatory activity was 0, 14, 43 and 86% respectively.

Ancillary Pharmacology Studies:

Progestational activity-

<u>Pregnancy maintenance:</u> Progestational activity of ZK 30595 (DRSP) was evaluated in pregnant rats after ovariectomy on day 8 of pregnancy. Pregnancy maintaining effect after a 4-day treatment was used as a parameter of progestational activity **(Study No.9879)**.

DRSP was administered on day 8 to day 11 of pregnancy either by SC route at dose levels of 0.1, 0.3, 1 and 3 mg/animal/day in 0.2 ml benzyl benzoate + castor oil (1+4) or as oral administration as either 1x3 mg every 24 hours, 2 x 1.5 mg every 12 hours, 3 x 1 mg every 8 hours or 6 x 0.5 mg every 4 hours in 0.5 ml vehicle (0.085% Myrj and 0.9% saline)

<u>Results:</u> 3 mg/animal/day SC dose maintained pregnancy by 80%, which was not different, compared to 89% for controls. Oral dose of 3 mg/animal/24 hours had no pregnancy maintaining effect. Dose divided in 2 x 1.5/animal/12 hours also had no effect. However, with 3 x 1 mg every 8 hours and 6 x 0.5 mg every 4 hours dosing schedule, pregnancy was maintained by 30% and 83%, respectively. Divided dosing was presumed to have improved blood level profile.

In study **Study No. A012**, 3 mg DRSP combined with 0.1 ug EE/animal/day had pregnancy maintaining effect of 92% which was similar to 93% for non-castrated control rats. However, lower and higher doses of EE diminished the degree of pregnancy maintenance (50% at 0.01 ug, 75% at 0.03 ug and 80% at 0.3 ug/animal/day. Pregnancy was not maintained (0%) when combined with 1 and 3 ug EE/animal/day, because of reported abortificiant activity of estrogen in the rat.

Antiandrogenic activity (Study No. A076): Juvenile Wistar male rats were castrated on day 1 of the experiment. From day 9-15 they were treated subcutaneously (s.c.) with 0.1 mg testosterone propionate (TP)/animal/day to stimulate the growth of accessory sex glands. Concomitantly DRSP and cyproterone acetate (ZK 9471) were administered orally (p.o) daily to antagonize the TP-induced organ growth. TP-controls were treated with TP (s.c.) and substance vehicle (p.o). Vehicle controls and non-castrated controls were treated with TP-vehicle (S.C.) and substance vehicle (p.o.). On day 16, the animals were killed and seminal vesicles, ventral prostate and adrenal glands were weighed.

Table 2. **Antiandrogenic activity of drospirenone**

Treatment group	Seminal vesicle mg/100g b.w.	% inhibition	Prostate weight Mg/100g b.w.	% inhibition	Adrrenal weight mg/100g b.w.
Non-castrated control	40.1+11.4	-	46.1 +7.6	-	24.4+2.7
Vehicle control	5.8 + 0.8	-	6.6 + 1.0	-	31.1 + 3.4
TP-control	42.5 + 7.5	-	36.0 +9.6	-	28.6 +4.4
DRSP 0.1 mg	41.0 +6.5	-4.2	31.1 + 4.6	-17.0	29.0 +2.0
0.3 mg	37.0 + 10.6	-15.1	29.5 +6.2	-22.2*	30.0 +3.7
1.0 mg	33.1 +3.0	-25.6*	25.5 +7.5	-35.7*	27.2 +4.1
3.0 mg	25.1 + 8.1	-47.5*	18.5 + 5.3	-59.6*	29.7 + 2.2
10 mg	13.2 +2.4	-79.8*	14.1 + 2.2	-74.5*	26.6 + 3.2
Cyproterone acetate 0.1 mg	33.1 +8.1	-25.7*	23.2 + 3.8	-43.7*	26.6 + 3.1
0.3 mg	24.6 + 7.5	-48.7 *	21.1 + 3.4	-50.9*	24.8 + 2.4
1.0 mg	14.8 + 4.7	-75.5*	14.8 + 3.4	-72.1*	21.7 + 3.0
3.0 mg	13.2 + 4.4	-80.0*	12.1 + 2.3	-81.3*	14.6 + 1.5
10 mg	8.1 + 1.2	-93.8*	10.4 +2.6	-87.0*	15.0 + 1.0

*=$p<0.05$ Doses are as mg/animal/day.

There were 6 animals in all groups except for vehicle control and TP-control where there were 12 animals. Values are mean+SD.

Results showed that DRSP exerts antiandrogenic activity in castrated and testosterone propionate treated juvenile male rats. DRSP was less potent in its antiandrogenicity than cyproterone acetate. Drospirenone had no effect on adrenal weight indicating that it lacks glucocorticoid activity.

The antiandrogenic effect of drospirenone was also observed in male rat fetuses after treatment of dams during days 17- 20 post coital (**Study No.A427**). The feminization effect of drospirenone compared to cyproterone acetate based on anogenital distance and length of urogenital sinus on day 22 post coital is shown in table below:

Table 3. **Antiandrogenic effect of DRSP in male fetuses**

Substance	n	Dose (S.C) mg/day/ animal	Anogenital distance In mm X+SD	Anogenital distance feminization vs control (%)	Length of UGS in mm x+SD	Length of UGS feminization vs control (%)
Drospirenone	13	10.0	1.5 + 0.4	67.6	3.2 + 0.8	72.4
	13	3.0	2.0 +0.3	35.3	4.3 + 0.6	30.7
	12	1.0	2.5 +0.3	1.5	4.9 + 0.3	9.8
	9	0.3	2.5 + 0.3	4.4	4.9 + 0.2	9.3
	12	0.1	2.3 + 0.3	14.9	4.9 +0.3	11.4
Cyproterone acetate	16	10.0	1.3 + 0.2	77.9	2.7 + 0.3	87.1
	12	3.0	1.8 + 0.4	49.3	4.0 + 0.5	42.6
	14	1.0	2.2 + 0.3	19.8	4.6 + 0.3	20.7
	12	0.3	2.4 + 0.2	8.2	5.0 +0.5	5.8
	11	0.1	2.3 + 0.2	16.5	4.8 + 0.3	13.7
Vehicle control male Fetuses	18	0.2 ml	2.6 + 0.4		5.2 + 0.3	
Vehicle control of female fetuses	21	0.2 ml	0.9 + 0.1		2.3 + 0.3	

N=number of fetuses inspected vehicle= benzylbenzoate + castor oil mix (1 + 4, v/v)

Results show that drospirenone has less antiandrogenic effects than cyproterone acetate.

Androgenic and antiandrogenic action of drospirenone was also determined in CV-1 cells stably transfected with the rat androgen receptor and MMTV-CAT reporter construct **(Study No. A276).**

In this transactivation assay, drospirenone showed no significant induction of CAT-activity (% CAT conversion) indicating no androgenic action compared to Metribolone (R1881, a synthetic androgen). CAT activity induced by R 1881 was inhibited by drospirenone. Compared to cyproterone acetate, drospirenone was less potent as an androgen.

Estrogenic potency of drospirenone (Study No. 9527). Ovariectomized Wistar adult female rats were injected SC with 10 mg of drospirenone and vaginal smears were examined 48, 54 and 72 hours post injection for proliferation of vaginal epithelium as a indicator of estrogenic potential.
The results are expressed as mean+SD:

	Dose mg/animal	Relative uterus weight (mg/100 b.w.) wet	Relative uterus weight (mg/100 b.w.) dry	Vaginal reaction
Control	-	49.3+4.3	10.0+0.9	no
Drospirenone	10	62.6+6.7*	10.7+1.2	no

*=<0.05

No effect of treatment on uterus dry weight and no effect on vaginal epithelium suggested no estrogenic effect of drospirenone.

Glucocorticoid and antiglucocorticoid potency of Drospirenone (study No. 9528): Thymolytic and antithymolytic affects of drospirenone were investigated in adrenalectomized male rats to evaluate its glucocorticoid and antiglucocorticoid potency. Cyproterone acetate, a progestagen with glucocorticoid activity and mifepristone, an antiprogestagen with antiglucocorticoid activity were used as reference substances.

Glucocorticoid potency was based on drug-induced suppression of thymus weight after a 4- day treatment. Doses of DRSP, cyproterone acetate and mifepristone were 10 mg/animal/day. The dose of dexamethasone, a reference standard with high glucocorticoid activity was 0.01 mg/animal/day.

For the determination of the antiglucocorticoid potency, animals were treated with dexamethasone at a dose of 0.01 mg/day for 4 days to suppress thymus weight. Simultaneously DRSP, cyproterone acetate or mifepristone were administered at doses of 1, 3 and 10 mg/animal/day to inhibit thymus weight suppression. The results were as follows:

Table 4. **Glucocorticoid and antiglucocorticoid activity of drospirenone**

	Suppression of thymus weight (%)	Glucocorticoid Potency	Inhibition of dexamethasone-induced thymus wt Suppression (%)	Antiglucocorticoid Potency
ZK 30595 (DRSP)	2.2	No	9.8	Marginal
Cyproterone acetate	44.3	High	9.9	Marginal
Dexamethasone	77.4			
Mifepristone 3 mg			15.9	Low
Mifepristone 10 mg	14.4	Marginal	48.3	High

Sponsor concluded that DRSP elicits no glucocorticoid activity and only marginal antiglucocorticoid activity in juvenile male rats. Doses for DRSP, cyproterone acetate and dexamethasone were 10 mg/animal/day. For mifepristone 3 and 10 mg/animal/day

Aldosterone antagonistic activity (Study No. 3906): For this study adrenalectomized, glucocorticoid-substituted Wistar rats were used. The study was based on the principle that the mineralo-cortical action can be induced in adrenalectomized rats through the exogenous administration of aldosterone i.e. sodium retention and K elimination and can be abolished through substances with aldosterone-antagonistic properties.

Aldosterone was infused continuously by i.v. and test substances (ZK 35973, a compound with structure similar to DRSP) and reference substances (spironolactone & DRSP) were given p.o. one hour before the start of infusion. Urine was collected in hourly fractions over the 6-hour experiment. Urine volume, Na^+ and K^+ concentrations were measured. Na^+/K^+ quotient and the log Na^+/K^+ x 100 were variables used as the basis of assessment.

<u>Results</u> of this study showed that drospirenone had significantly stronger aldosterone-antagonistic action compared to spironolactone.

In study **No.3435**, using a similar experimental design as in the above study, DRSP was reported to be a more potent and longer-acting aldosterone-antagonistic substance than spironolactone.

Under **study No. 5995/II**, the ability of 11 steroids with varying degrees of progestogenic potency to exhibit the renal actions of aldosterone was tested in adrenalectomized glucocorticoid-treated rats. The study design was the same as in **study No.3906**. Comparison of progestogenic and antimineralocorticoid activity of 9 steroids is shown in table below:

Table 5. Comparison of progestogenic and antimineralocorticoid activity of 9 steroids

	Receptor binding	progestogenic activity: inhibition of ovulation	progestogenic activity: maintenance of pregnancy after ovariectomy	antimineralocorticoid activity
	progesterone=1	effective threshold dose mg/rat	effective threshold dose mg/rat	effective threshold dose
Compound		s.c/orally	s.c./orally	s.c./orally
Progesterone	1.0	1/>10	3/>10	2/>16
Cyproterone acetate	0.78	0.3/1	1/<10	>8/n.t.
Norethisterone acetate	0.33	0.3/3	0.3/n.t.	>8/n.t.
3-ketodesogestrel	1.67	0.01/n.t.	0.01/>3	>8/n.t.
D-norgestrel	1.25	0.03/3	0.03/10	>8/n.t.
Gestodene	1.25	0.003/n.t.	0.003/>10	4/>8
Spironolactone	0.05	40/30	n.t.	1/1
Spirorenone	0.15	>10/n.t.	>3/30	<0.5/<0.5
1,2-dihydrospirorenon (drospirenone)	0.40	0.3/n.t.	0.3/>3	<1/0.25

Receptor binding was conducted using rabbit uterus cytosol expressed as relative binding affinity with progesterone=1. n.t.=not tested All steroid were administered by s.c. route. The data is a compilation from various studies.
With the exception of progesterone, the antimineralocorticoid activity of the other steroids could be demonstrated after oral administration. While cyproterone acetate, norethindrone acetate, 3-keto desogestrel and norgestrel did not show antimineralocorticoid activity when administered s.c. progesterone, gestodene, spirorenone and drospirenone exhibited significant antimineralocorticoid activity.

Ethinyl estradiol did not interfere with the mineralocorticoid-antagonistic effect of drospirenone in ovariectomized-adrenalectomized Wistar rats (**Study No. 9995**).

In **study No. A085**, it was shown that drospirenone but not spironolactone is a potent antimineralocorticoid under long term treatment with a dose of 10 mg/animal/day. Sponsor attributed this to the fact that the counter-regulation with stimulated aldosterone levels does not overcome the antialdosterone effect of drospirenone but does so for spironolactone.

Antigonadotropic activity (Study No.5972): Antigonadotropic activity of Metispirone, spironolactone and DRSP was determined in sexually mature male cynomolgus monkey model. The potency of the test substance was based on a decrease in serum concentrations of testosterone and LH.

Because of the wide variation of the pre-dosage testosterone and LH values, the threshold dose was determined as an index of antigonadotropic activity. The threshold dose was defined as the lowest dose that will lower serum testosterone and LH concentrations to below control or pre-dosage levels within treatment period of 11 days.
By oral daily administration, the threshold dose was as follows:

Metispirone	40 mg/kg
Spironolactone	80 mg/kg
Drospirenone	2-4 mg/kg

Steroid hormone receptor binding studies for DRSP (Study No. 9691): Receptor binding was studied in cytosolic fractions of steroid-dependent tissues by competitive protein binding in presence of the appropriate tritiated steroids. The activity of a compound is indicated as a competition factor (CF), which is defined as the multiple of the concentration needed to obtain displacement equivalent to the reference compound.

Table 6. **Drospirenone receptor binding**

Receptor Reference Compound	Progestogen Progesterone=1		Mineralocorticoid Aldosterone=1		Androgen DHT=1	Estrogen Estradiol=1		Glucocorticoid Dexamethasone=1
Species Tissue	Rabbit Uterus	human uterus	rat kidney	rat hippocampus	Rat Prostate	rat uterus	human uterus	Rat Thymus
CF DRSP	2.5	2.2	0.6	1.0	170	Did no bind to either		103

Relative binding affinities (RBA) to human steroid hormone binding protein (h SHBP) and corticoid binding globulin (CBG) in charcoal stripped pregnancy serum was as follows:

Compound	SHBG (DHT=100) RBA	CBG (cortisol=100)
DRSP	1.6	<0.01

From these receptor binding affinities studies, sponsor concluded that drospirenone has progestogenic activity, antimineralocorticoid action, exerts no detectable androgenic activity, and exerts low glucocorticoid activity.
It was stated that "in contrast to the progestogens levonorgestrel, gestodene and 3-ketodesogestrel, which exhibit considerable binding affinity to SHBG, DRSP exerts low affinity to SHBG. In this respect drospirenone behaves again similar to progesterone."

Under neutral environmental conditions DRSP is converted to 17-a-spiro-isomer of DRSP. Therefore, the receptor profile and pharmacological actions of the isomer was tested concerning progestogenic, antiandrogenic and antimineralocorticoid activities (**Study No.A370**).

Results: Steroid binding assays showed a very low binding affinity to the progesterone and androgen receptor with competition factor of 170 for progesterone receptor from uteri of estrogen substituted rabbits and castrated rats prostate receptor, respectively. Affinity for mineralocorticoid receptor was not tested. However, urinary Na^+/K^+ ratio for aldosterone control and aldosterone + 4 mg p.o. 17-a-spiro-isomer had similar effects i.e. exhibited no antimineralocorticoid effect. The isomer was thus considered not a hormonally active compound. Clinical implications of DRSP isomerization was not discussed.

Summary of pharmacology: Drospirenone's contraceptive action is attributed to its suppression of gonadotropins. Although the primary mechanism of this action is inhibition of ovulation, other alterations include changes in the cervical mucus (which increases the difficulty of sperm entry into the uterus) and the endometrium (which reduces the likelihood of implantation). Drospirenone has been shown to have progestogenic, antimineralocorticoid and antiandrogenic activities. It exerted no detectable androgenic, estrogenic or glucocorticoid activities. Drospirenone under neutral environmental conditions isomerises to 17-a-spiro-isomer, which was reported to be not hormonally active compound.

SAFETY PHARMACOLOGY:

Neurological effects:

The effect of drospirenone after single oral pretreatment on the hexobarbital-induced loss of righting reflex was determined in mice **(Study No. AJ95).** Ninety minutes after oral pretreatment, animals received the barbiturate i.v. and sleeping time was recorded.
It was reported that single oral pretreatment with drospirenone at doses up to 100 mg/kg or with EE at doses up to 1 mg/kg did not result in a dose-related prolongation of hexobarbital sleeping time.
Combination of drospirenone with EE at a ratio of 100:1 resulted in a dose-dependent prolongation of the CNS depressant action of hexobarbital; the ED_{50} was 98.3 mg/kg p.o.

Neurotropic effects of drospirenone alone or in combination with EE was determined after single oral administration in female mice (**Study No.AJ90**). Drospirenone was used at doses up to 800 mg/kg and EE at up to 8 mg/kg.

Results: Single oral administration of drospirenone at doses greater than 400 mg/kg caused neurotropic effects (isolated stimulatory signs, decreased locomotor activity and gait alterations) which did not last beyond 4 h post treatment.

400 mg/kg drospirenone with 4 mg/kg EE caused a pattern of neurotropic effects identical to those produced by drospirenone alone at doses greater than 400 mg/kg.
EE had no appreciable neurotropic effect at doses up to 8 mg/kg dose.

Cardiovascular effects:

The ability of gestodene, 3-Ketodesogesterel (3-KDSG) and drospirenone to induce hypertension in comparison to the mineralocorticoid deoxycortone acetate (DOCA) was investigated in ovariectomized, estrogen-substituted female rats under conditions which promote the development of such mineralocorticoid hypertension (**Study No. 9806**).

Table 7. **Mean arterial blood pressure in conscious rats with various progestogen treatments**

	Duration of treatment (weeks)				
	0	1	2	3	4
DOCA vehicle	119 + 7 (10)	121 + 9 (10)	131 + 18 (8)	124 + 13 (6)	132 + 14 (6)
DOCA	119 + 8 (11)	134 + 22 (11)	142 + 16 (11)	149 + 15 (11)	149 + 22 (9)
Progestogen vehicle	121 + 9 (9)	124 + 10 (8)	124 + 16 (5)	126 + 11 (6)	132 +19 (6)
Gestodene	120 + 5 (9)	125 + 8 (9)	124 + 8 (9)	124 + 8 (9)	122 + 5 (6)
3-Keto-desogestrel	122 + 8 (9)	127 + 16 (8)	124 + 6 (7)	121 + 12 (7)	123 + 15 (3)
DRSP	121 + 6 (10)	120 + 9 (100	123 + 9 (10)	121 + 5 (9)	122 + 7 (10)

Progestogen doses represented a maximum effective dose given s.c. for the inhibition of ovulation for each of the compounds. DOCA was given at a dose of 1.5 mg/100 g body weight s.c. twice weekly. Gestodene and 3-KDSG were given at a dose of 0.01 mg/animal and DRSP at a dose of 3 mg/animal s.c.once daily.

Results: It was stated that multivariate analysis over the experimental period showed a significant difference between DOCA vehicle and DOCA, but not between any of the progestogens and their respective vehicle controls.

Effects on blood pressure and heart rat in normotensive female rats by daily s.c. administration of drospirenone (1 and 10 mg/animal/day) and progesterone (3 and 30 mg/animal/day) for 21 days was determined in **Study No. AD90**. Doses used were based on their ability to show progestogenic activity i.e. maintenance of pregnancy and inhibition of ovulation. Reference substance was progesterone and was used at a dose, which exerts comparable pharmacological profile as drospirenone. Telemetry was used for continuous blood pressure measurements in unrestricted animals.

Results:

<u>Drospirenone:</u> Systolic blood pressure in rats was reduced by 2-3 mmHg by 1 mg dose compared to vehicle controls. The 10 mg dose reduced systolic blood pressure by 4 mmHg. Diastolic blood pressure and heart rates were not affected by either dose.

<u>Progesterone:</u> Compared to vehicle controls, 3 but not 30 mg/animal/day significantly increased diastolic BP by 3.5 mmHg. Systolic BP was decreased by 2-3 mmHg by 30 mg dose. Heart rate was increased by 10-15 beats/min by both doses compared to vehicle controls.

Under similar experimental conditions as in **Study No. AD90**, 10 mg/animal/day of drospirenone (**Study No.AD89**) reduced systolic BP by 5.6 mmHg. The 1 mg dose had no effect. Neither dose affected diastolic BP or heart rate.

Progesterone at 3 but not at 30 mg/animal/day significantly increased diastolic BP pressure by 6.9 mmHg at the end of the experiment. Systolic BP and heart rate were not significantly affected by either dose.

The effects of deoxycortone acetate, gestodene, DRSP and 3-ketodesogestrel on vascular contraction were investigated using the rat's tail artery (**Study No 9812**).
The study was designed to investigate the effects of steroids possessing mineralocorticoid or antimineralocorticoid activity, on vascular reactivity in the rat-tail artery. Vasoconstriction was induced by the exogenous and endogenous vasoconstrictor agents, methoxamine and noradrenaline, respectively. Endogenous transmitter was released by transmural nerve stimulation (TMNS).
The experimental model used was ovariectomized, uninephrectomized, ethinylestradiol substituted female rat.

Results: It was reported that 4 week pre-treatment with gestodene (0.01 mg/day), DRSP (3 mg/day), 3-ketoDSG (0.01mg/day) or DOCA (1.5 mg/100 g b.w. twice weekly) did not influence the vascular reactivity to exogenously applied a_1 adrenoceptor agent, methoxamine. Also treatment did not appear to affect vasoconstriction produced by release of endogenous transmitter noradrenaline by TMNS.

Pulmonary effects:

Influence of drospirenone and DRSP + EE on pulmonary function, various CV parameters, motility and intraluminal pressure in the uterus and intestine of anesthetized rabbits was determined after i.v. infusion. (**Study No.AP26**).

Pulmonary function parameters tested were respiratory frequency, tidal volume, intrapleural pressure, resistance and compliance. CV parameters consisted of blood pressure, heart rate, blood flow and ECG. Uterine and intestinal parameters included motility and intrauterine pressure and intestinal motility and intraluminal intestinal pressure.

Drospirenone was used at doses of 60 and 600 ug/kg alone or in combination with EE in ratio of 100:1.

Results: Significant treatment-related changes were decreased pulmonary intrapleural resistance with the low dose of drospirenone alone. ECG-QRS interval was decreased with the low dose of drospirenone alone as well as with EE, which was not observed with the high dose of drospirenone alone or with EE. The high dose combination of drospirenone with EE increased the intrauterine pressure and the strength of uterine contractions.

Renal effects: Even though DRSP has been shown to have strong anti-mineralocorticoid effects, its affect on renal function was not investigated.

Gastrointestinal effects: DRSP was reported to have no effect on intestinal intrpleural pressure. (StudyNo. AP26).

Abuse liability: None described.

Other effects: The effect of drospirenone alone or in combination with EE on smooth muscle was determined using isolated guinea pig uterus horn, ileum and trachea (**Study No.A551**). At concentration of 10^{-5} mol/l, drospirenone had no effect on smooth muscle from any organ. While treatment with drospirenone at doses up to 100 mg/kg or EE at doses up to 1 mg/kg individually did not influence rectal temperature in mice, 100 mg/kg drospirenone in combination with 1 mg/kg EE significantly lowered rectal temperature at 120 min post treatment (**Study No. AJ91**).

Summary: Drospirenone at oral doses up to 400 mg/kg had no effect on hexobarbital sleeping time in mice. Doses greater than 400 mg/kg caused neurotropic effects.
Treatment of rats resulted in a slight decrease in systolic blood pressure but had no effect on diastolic pressure or heart rate.
Low doses of drospirenone decreased pulmonary intrapleural resistance. Drospirenone had no effect on smooth muscle.

PHARMACOKINETICS/TOXICOKINETICS:

Since no statement was made regarding GLP regulations, it is presumed that PK studies reviewed below were non-GLP.
PK parameters: The PK parameters of DRSP and EE in female mice after single as well as single and multiple (14 days) intragastric administration are summarized in the following 2 tables (**Study No. A705**).

Table 8. **Pharmacokinetic parameters of DRSP in female mice**

Parameter	Unit	Dose (mg/kg)						
		1.0 (s.i.v)	3.0 (s.i.g)	10.0 (s.i.g.)	30.0 (s.i.g.)	3.0(m.i.g.)	10.0 (m.i.g.)	30.0 (m.i.g.)
C_{max}	ng/ml	493	60.7	532	1922	48.8	671	2408
t_{max}	h	0.08	0.5	0.5	0.5	0.5	0.5	0.5
$T_{1/2}$	h	1.1	1.7	1.4	1.5	1.5	1.6	1.5
AUC (0-4h)	ng.h/ml	164	73	546	4494	67	757	6039
AUC(0-16 ?)	ng.h/ml	165	89	627	6522	85	893	8401
MRT	h	0.3	2.2	1.7	2.7	2.1	1.9	2.6
CL	ml/min/kg	101	n.d.	n.d	n.d.	n.d.	n.d.	n.d.
V_z	l/kg	9.68	n.d	n.d	n.d	n.d	n.d	n.d
V_{ss}	l/kg	1.72	n.d	n.d	n.d	n.d	n.d	n.d
Bioavailability		100	18%	38%	132%	17%	54%	170%
AUC_{TX}/ AUC_{HU}			0.06	0.4	4.1	0.05	0.6	5.3

In a human study (PhRR A 470) a mean DRSP-AUC of 1589 ng.h/ml (AUC_{HU}) was calculated after multiple daily administration of the anticipated oral contraceptive dose (3 mg DRSP + 30 ug EE_2/inndividual/day) to young, healthy women.

Table 9. **Pharmacokinetic parameters of EE in female mice**

Parameters	Unit	Dose (mg/kg)				
		1.0 (s.i.v.)	0.1 (s.i.g.)	0.3 (s.i.g.)	0.1 (m.i.g.)	0.3 (m.i.g.)
C_{max}	pg/ml	49660	320	1944	220	1219
t_{max}	h	0.08	0.5	0.5	0.5	0.5
$T_{1/2}$	h	n.d	n.d.	n.d.	n.d.	n.d.
AUC (0-4h)	pg.h/ml	14556	565	1756	427	1418
AUC (0-16h)	pg.h/ml	14814 •	1098	2596	846	2201
MRT	h	0.5	10.0	4.4	8.6	5.1
CL	ml/min/kg	112	n.d.	n.d.	n.d.	n.d.
V_z	l/kg	14.4	n.d.	n.d.	n.d.	n.d.
V_{ss}	l/kg	3.03	n.d.	n.d.	n.d.	n.d.
Bioavailability		100%	7%	6%	6%	5%
AUC_{TX}/ AUC_{HU}			0.9	2.1	0.7	1.8

s.i.v.=single intravenous; s.i.g.=single intragastric; m.i.g.=multiple intragastric
It was stated that limited toxicological parameter determined revealed no signs of drug-related adverse effects

The PK data demonstrate:

- Absorption of both DRSP and EE is rapid
- PK is not altered by multiple intragastric administration
- DRSP and EE do not accumulate on multiple intragastric administration
- Absolute bioavailability is high for DRSP but low for EE
- The metabolic clearance indicates the contribution of extrahepatic metabolism
- Systemic exposure for DRSP was not dose proportional

Pharmacokinetics of the unchanged drug and the ^{14}C-radioactivity after single intragastric administration of 1 mg/kg and 10 mg/kg and single intravenous administration of 0.66 mg/kg ^{14}C-DRSP to female rats was investigated in **study No. 9518.**
Plasma samples were collected up to 48 hours post administration. Plasma concentration of DRSP was determined by means of a specific radioimmunoassay whereas the plasma concentration of ^{14}C-radioactivity was determined by liquid scintillation counting.
The PK parameters of DRSP and those of radioactive substances are shown in table below:

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Table 10. **PK parameters of DRSP in female rats**

	PK parameters of DRSP			PK parameters of radioactive substances		
Dose Route of administration	0.66mg/kg i.v	1 mg/kg i.g.	10 mg/kg i.g.	0.66 mg/kg i.v.	1 mg/kg i.g.	10 mg/kg i.g.
C_{max} (ng/ml) C_{max} (ng-equiv/ml)	102	82	727	549	363	4360
T_{max} (h)	0.25	1.25	1.22	0.25	1.10	1.34
$T_{1/2}$ (h)	3.2	3.2	2.3	3.3	3.5	2.9
AUC (ng.h/ml) AUC (ng-equiv.h/ml)	343	346	5230	1970	1970	29900
MRT (h)	3.4	4.5	5.3	4.1	5.0	6.0
CL(ml/min/kg)	32.0	n.d.	n.d.			
V_z (l.kg)	8.77	n.d.	n.d.			
V_{ss} (l/kg)	6.53	n.d	n.d.			
Bioavailability (%)	100	67	101			

Since the radioactive compounds in plasma decreased approximately with the same rate as the unchanged drug, it was implied that the metabolites of DRSP could not have long terminal half-lives. Also since after both routes of administration, the levels of the radioactive compounds in plasma were about 6-fold higher than the corresponding concentrations of the unchanged drug, it was suggested that 80% of the radioactivity present in plasma represented metabolites of DRSP.

The AUC (0-24h) $_{plasma}$/AUC (0-24h)$_{blood}$ for the 0.66 mg/kg i.v. dose and for the 1 and 10 mg/kg i.g. dose was 1.03, 0.97 and 1.10 respectively, indicating the drug was equally distributed between blood cells and plasma.

Plasma levels, tissue distribution and excretion of the unchanged drospirenone after single i.v. (1 mg/kg) or i.g (1 or 10 mg/kg) was tested in female rats (**Study No. A154**).

At different times up to 2 days after administration, 3 animals were sacrificed for blood collection. In addition, liver, brain and pituitary gland were removed at 0.25, 2, 6 and 24 hours. The concentration of DRSP in plasma, urine, feces and tissue extracts was determined by RIA. Values are mean + S.D.

Table 11. **PK parameters of DRSP In female rats**

		Dose		
Parameter	unit	1 mg/kg i.v.	1 mg/kg i.g	10 mg/kg i.g.
C_{max}	ng.ml	281 + 117	112 + 9	1130 + 94
t_{max}	h	0.8 + 1.0	0.4 + 0.1	2.7 + 1.5
$T_{1/2}$	h	4.0	4.7	3.1
MRT	h	4.4	5.1	6.0
AUC	ng.h/ml	747	495	7919
Cl	ml/min/kg	22.3	-	-
V_z	l/kg	7.7	-	-
V_{ss}	l/kg	5.9	-	-
Bioavailability		100	70	110

V_z= volume of distribution during the terminal disposition phase. A value of 7.7 indicated that the drug is widely distributed in the tissue.

After both i.v. and i.g. administration the concentration of DRSP in the liver was at least 7 times higher than in plasma, suggesting liver as the main organ of drug metabolism and excretion. The concentration of DRSP was generally higher in brain compared to plasma indicating that the drug passes the blood brain barrier. The concentration of unchanged DRSP in the pituitary was similar to that in plasma up to 2 hours but then increased up to 16 times higher, suggesting delayed elimination from this tissue.
Independent of the dose and route of administration, only less than 1% was excreted in the urine.

Note: Sponsor reported that after the completion of this study, they found that DRSP is not stable in plasma of mice, rats and rabbits. Incubation of DRSP in rat plasma resulted in a reduction of drug concentration by 50% within 3 hours of incubation. This was suggested to be due to esterase activity affecting the lactone ring. Sponsor stated that the new metabolite was not yet identified.

To prevent enzymatic conversion of DRSP in plasma on storage, PK study was repeated (**Study No. AF68**) where an esterase inhibitor, 4-(2-aminoethyl)-benzene sulfonyl fluoride hydrochloride (PEFABLOC SC) was added to blood samples immediately after withdrawal in order to prevent the decay of the drug substance in the plasma samples before analysis. The results are shown in table below:

Table 12. **PK parameters of DRSP in female rats for samples with esterase inhibitor**

PK parameter	Unit	1 mg/kg i.v.	1 mg/kg i.g.	10 mg/kg i.g.
Cmax	ng/ml	393.9	162	1440
Tmax	h	0.08	1.5	2.0
T1/2	h	6.5	2.6	3.0
AUC	ng.h/ml	517	510	8641
MRT	h	3.2	3.1	5.5
Cl	ml/min/kg	32.3	n.d.	n.d.
V_z	L/kg	18.0	n.d.	n.d.
V_{ss}	L/kg	6.1	n.d.	n.d.
Bioavailability	%	100	99	167

Values are mean of 3 rats. S.D. was not given.

Note: Addition of enzyme inhibitor did not seem to significantly affect Cmax or AUC compared to those reported under **Study No. A154** using the same dosage levels.

PK of one i.v. and two i.g. administration of EE was determined in female Wistar rats (Study No. AB03). Each group comprised of 36 animals. Doses used were 0.1 mg/kg for the i.v. administration and 0.1 and 0.03 mg/kg for the i.g. administration. Three rats were sacrificed at various time points up to 24 hours for blood collection. PK parameters expressed as mean + S.D. is shown below.

Table 13. PK parameters of EE in female rats

Parameter	0.1 mg/kg i.v.	0.1 mg/kg i.g.	0.03 mg/kg i.g.
Cmax (pg/ml)	66200	411	302
Tmax (h)	0.08	1.0	0.5
AUC (pg.h/ml)	22563	2240	958
T1/2 (h)	10.4	5.6	8.6
Cl (ml/min/kg)	73.9	73.7	74.1
V_{SS} (l/kg)	6.7	35.4	26.7
Bioavailability %	100	9.9	14.2

Drug was rapidly absorbed, underwent extensive first-pass effect and had low bioavailability.

Pharmacokinetic parameters of unchanged DRSP were determined in rabbit after single i.v. and single i.g. administration (**Study No. 9392**). Five rabbits were used. They first received a single intragastric administration of 0.5 mg/kg DRSP, followed 2 weeks later by a single intragastric administration of 5 mg/kg. Three weeks after the second dose, the animals received a single intravenous administration of 0.5 mg/kg. The results expressed as mean + S,D. is shown in table below:

Table 14. **PK parameters of DRSP in rabbits**

PK parameter	0.5 mg/kg i.v.	0.5 mg/kg i.g.	5 mg/kg i.g.
Cmax (ng/ml)	203.5 + 71.5[c]	31.7 +18.4	147.2+86.4
Tmax (h)	0.19 + 0.21[c]	1.2 + 0.4	2.5 + 1.1
T1/2 (h)	0.7 + 0.2	0.9 + 0.1[a]	2.9[b]
T1/2 (h)	6.9 + 4.3	5.9 + 1.2[b]	5.9 + 0.3
AUC (ng.h/ml)	269 + 102	105 + 32[c]	993 + 312
Cl (ml/min/kg)	35.0 + 13.7	33.1 + 16.0	34.6 + 14.2
V_Z (l/kg)	19.7 + 10.0	18.0 +12.2	17.8 + 7.6
V_{SS} (l/kg)	10.5 + 9.0	n.d.	n.d.
MRT (h)	4.5 + 2.0	6.8 + 3.2	7.2 + 2.1
Bioavailability		43 + 22[c]	43 + 26

A: n=2 b: n=1 c: n=4

Results thus showed that compared to rats and mice, the bioavailability of DRSP was low in rabbits.

Note: sponsor did not indicate if the esterase inhibitor was used in this study. However, in annual report dated 9-27-1999, it was stated that esterase inhibitor, ethylene glycol-bis (B-amino-ethylether)-N,N'tetraacetic acid (EGTA) was used in a study entitled "Ex vivo stability of drospirenone in plasma samples of different species".

Under the annual report, the following summary table for PK of DRSP in female rabbits following single i.v. injection of 1 mg/kg and single i.g. administration of 1, 10 and 100 mg/kg DRSP was provided:

Table 15. **PK of DRSP in female rabbits**

	1 mg/kg(oral)	10 mg/kg(oral)	100 mg/kg(oral)	1 mg/kg (iv)
Cmax (ng/ml)	38.0 + 9.5	415 + 129	1866 + 407	465 + 29.3
Tmax (hours)	0.5	0.7 + 0.3	4.7 + 1.2	0.25
T1/2 (hours)	3.9 + 0.8	4.9 + 0.8	5.5 + 3.5	3.2 + 0.3
AUC 0-) ng.hr/ml	185 + 3.38	1670 + 456	24423 + 5389	870 + 261
AUC (0-24h) ng.hr/ml	182 + 3.30	1623 + 425	23995 + 5398	868 + 262
MRT (hours)	4.4 + 0.2	4.5 + 0.5	5.6 + 0.7	2.6 + 0.2
Bioavailability	21%	19%	28%	N.A.

- Number of rabbits/g was not given. Results showed that the bioavailability is low in rabbits compared to rats and mice.

Plasma level of unchanged drug after a single oral administration of 1 mg DRSP as a normal tablet (SH T 470 A) and as a film-coated tablet resistant to gastric juice (SH T 470 B) was determined in 6 female beagle dogs **(Study No. 7797)**

It was reported under study No. 4627, that DRSP undergoes acid-catalyzed decomposition and rearrangement.
The objective of the study was to establish whether the bioavailability of DRSP was impaired by inactivation in the acid milieu of the stomach, by determining the plasma level of the unchanged drug.

The animals were divided in 2 groups of 3 animals each. Dogs of group 1 received tablet A and after a washout period of 3 week, a tablet B. The medication was given in a reverse order in group 2. PK parameters expressed a mean + S.D. is shown in table below:

Table 16. **Effect of acid milieu of stomach on DRSP bioavailability**

	Group 1	Group 2
Parameter	1 mg SH T 470 A n=6	1 mg SH T 470 B n=4
Cmax (ng.ml)	41 + 17	46 + 10
Tmax (h)	2.0 + 0.7	2.8 + 1.0
T1/2 (h)	3.3 + 0.6	3.0 + 0.5
Time interval (h)	6-28	3-28
T1/2 (h)	37 + 10	36 + 11
Time interval (h)	28-96	28-96
AUC (ng.h/ml)	213 + 40	223 + 47
AUC 0- (ng.h/ml)	219 + 42	229 + 44
Rel. bioavailability (%)	97	100

Two of the 6 dog in group 2, displayed incomplete absorption The Cmax values of 2.0 and 2.7 ng/ml were not measured until after 8 and 48 hours, respectively. The absolute bioavailability was about 80% considering AUC value of 854 + 70 ng.h/ml after 3 mg i.v.dose.

Results showed that in the dog adequate bioavailability could be achieved with tablets without a coating resistant to gastric juice. Sponsor concluded that coating appears unnecessary. It was however, nor stated as to what extent DRSP was isomerized to its acidic form i.e. ZK 35096, which was reported to be not hormonally active compound.

Under **Study No. 9037,** plasma level and excretion of the unchanged drug in urine and feces after single intravenous and single intragastric administration of DRSP were determined in 5 female cynomolgus monkeys.

The study was designed as an intra-individual comparison of one i.v. and two i.g. administrations. Blood, urine and feces were collected up to 3 days after administration.

Table 17. **PK parameters in monkeys expressed as mean + S.D.**

Parameter	0.5 mg/kg i.v.	1 mg/kg i.g.	10 mg/kg i.g.
Cmax (ng/ml)	375 + 71	368 + 147	1846 + 1025
Tmax (h)	0.23 + 0.16	2 + 1	3 + 1
T1/2 (h)	11.4 + 5.1	10.8 + 8.3	19.6 + 4.0
AUC (ng.h/ml)	2822 + 1060	4060 + 1978	30367 + 18314
MRT (h)	13.4 + 6.7	13.0 + 8.7	28.3 + 3.1
Cl (ml/min/kg)	3.3 + 1.1	-	-
V_z (l/kg)	3.0 + 1.1	-	-
V_{ss} (l/kg)	2.4 + 0.8	-	-
Bioavailability %	-	74 +30	55 +9

Unchanged drug excreted in urine and feces was than 1.5% and 3.3% of the administered dose, respectively.

Based on only minor amounts of unchanged drug being excreted in urine and feces, it was implied that drug was completely metabolized in the body. Also based on longer terminal half-life of the unchanged drug with high dose compared to low dose (20 vs 11 hours), it was suggested that at most 2-fold accumulation of the drug could be expected after repeated, once daily administration

Under **Study No. A376**, ^{14}C-drospirenone was administered as a single i.v. or i.g. dose as in **Study No. 9037** to 4 female cynomolgus monkeys. Blood samples were taken up to 96 hours post -dosing. PK parameters of drospirenone were essentially similar as observed in **Study No. 9037**.

Table 18. **PK parameters of ^{14}C labeled substances as ^{14}C-radioactiviy**

	Route of administration		
Parameter	Intravenous (o.5 mg/kg)	Intragastric (1 mg/kg)	Intragastric (10 mg/kg)
Cmax (ng-equiv./ml)	304 + 100	455 + 45.5	3927 + 398
Tmax (h)	0.25 + 0	2.1 + 1.0	3.9 + 0.9
T1/2 (h)	33.7 + 4.7	29.5 + 2.6	32.2 + 4.6
AUC (ng-equiv.h/ml)	9885 + 1713	13255 + 2970	98877 + 17580

Data showed that absorption was faster at the lower dose. Values are mean + S.D.
Although PK parameters of drospirenone are not shown here, it was observed that the elimination of unchanged drug was 2-3 times faster from the plasma than the labeled compounds, indicating metabolites had longer terminal half-life than DRSP. The ratio of concentrations of labeled compounds in plasma and whole blood ranged from 1.39-1.74, indicating unequal distribution.

In a dose range finding study in 3 female cynomolgus monkeys (**Study No. 9912**), a single i.g. dose of 0.3 mg drospirenone/kg with 20, 40 or 80 ug of EE resulted in dose-dependent increase in EE systemic exposure. EE did not influence systemic exposure to drospirenone.

Transplacental transport of DRSP and its metabolites was determined after a single i.v. (o.5 mg/kg) and single i.g. (0.6 mg/kg) administration of ^{14}C-DRSP to pregnant rabbits on day 18 or day 19 post coitum **(Study No. A763).**

Results of this study expressed as % (mean of 3 animals) of the dose administered recovered in various tissues at 4 time points is shown in table below.

Table 19. **Transplacental transport of DRSP in rabbits**

intravenous

Time (h)	Placenta	uterus	Liver	Carcases	Fetal liver	blood	plasma	Amniotic fl
0.25	0.33	0.76	22.51	0.187	0.036	5.24	5.13	0.017
1.5	0.16	0.68	12.63	0.064	0.010	3.50	3.47	0.015
6	0.06	0.19	4.61	0.020	0.003	0.89	0.89	0.008
24	0.01	0.04	1.06	0.002	0.001	0.12	0.12	0.002
Intragastric								
0.5	0.04	0.08	4.59	0.007	0.001	0.54	0.57	0.001
1.5	0.03	0.12	3.56	0.007	0.001	0.64	0.68	0.004
6	0.03	0.19	3.54	0.006	0.001	0.57	0.59	0.005
24	0.01	0.03	0.84	0.004	0.001	0.08	0.08	0.002

Results showed that DRSP and its metabolites do cross the placental barrier after both i.v. and i.g. administration. The highest amount of labeled substances recovered in fetuses were calculated to be about 0.23% of the dose after i.v. and less than 0.01% of the dose after i.g. administration.

Absorption: Absorption of drospirenone was rapid in all species tested.

Distribution: After both intravenous and intragastric admininstration, drospirenone concentration was highest in the liver. Drospirenone crosses the blood-brain barrier.

Metabolism: Metabolism of DRSP in mice, rats, rabbits and monkeys was investigated under **Study No. AY62** using ^{14}C-drospirenone. Concentrations of DRSP and its metabolites (ng.equiv./ml) were determined after single i.g. administration of 1, 4.8 or 10 mg ^{14}C-DRSP/kg in rats, 10 mg/kg in mice and 1 or 10 mg/kg in monkeys. In the rabbit it was determined after a single i.g. administration of 0.6 mg or 5 mg DRSP/kg and single i.v. administration of 0.5 mg ^{14}C-DRSP/kg. Concentrations of DRSP and metabolites were calculated from radiochromatograms peaks for 6 rats, 4 rabbits and 3 monkeys. Plasma samples were pooled for various time intervals.

Table 20. **Concentrations of DRSP and metabolites in the plasma of rat, monkey and rabbit**

Data for rat plasma after a single 10 mg oral dose

Sample	PPM	M11	DRSP
Pool 1 h	117	818	661
Pool 4 h	41	578	355
Pool 8 h	38	226	174

Values are expressed as ng-equivalents/ml

Data for monkey plasma after a single 10 mg oral dose

Sample	PPM	M3a	M11	M14	M16	M17	DRSP
Pool 2-4 h	182	n.d.	291	130	218	205	1080
Pool 8 h	228	62	196	99	139	344	525
Pool 24 h	240	49	43	55	41	101	147

Values are expressed as ng-equivalents/ml

Data for rabbit plasma after a single 0.6 mg oral or 0.5 mg i.v. dose

Sample	PPM	M9	M11	M14	M16	DRSP
Rabbit plasma pool i.g. 0.5-6 h	32	7	9	3	8	4
Rabbit plasma pool i.v. 0.25-6 h	39	14	48	8	21	14

Values are expressed as ng-equivalents/ml

Data for mouse serum after a single intragastric dose of 10 mg/kg

Sample	PSM	M9	M11	M14	M16	M17	DRSP
Pool 0.5 h	76.8	49.9	64.0	182	163	83.7	2o8
Pool 2 h	83.5	23.5	61.6	115	61.0	69.7	156
Pool 4h	86.8	n.d.	34.1	79.0	83.0	46.8	72.8

Values are expressed as ng-equivalents/ml

PPM=polar plasma metabolites. PSM=Polar serum metabolites. Values are expressed as ng-equivalents/ml

M 11 (acid form of DRSP generated by hydrolysis of the carbolactone ring), M 14 (4,5-dihydro DRSP-3-sulfate)

Sponsor concluded that metabolite profiles in the rat, mouse, rabbit and monkey plasma were qualitatively similar and were similar to metabolite profile in human plasma. The major human metabolites, M11 (free acid form of DRSP generated by opening of the lactone ring) and M14 (4,5-dihydrodrospienone-3-sulfate) were identified in the plasma of rats, mouse, rabbits and monkeys, however, M14 was only available in trace amounts in rats and rabbits. Qualitative as well as quantitative differences in metabolite pattern was observed in the urine and feces of mice, rats, rabbits and monkeys. Urine samples of rats and rabbits showed a similar metabolite pattern with a major peak corresponding to M11 with regards to the retention time. Sponsor stated that due to the complexity of the human metabolite pattern in urine and feces, individual peaks were difficult to assign in human samples.

Elimination: The excretion of ^{14}C-labelled compounds (% of dose) in urine and feces after a single intragastric and intravenous administration of ^{14}C-DRSP in female mice, rats, rabbits and monkeys was determined under Study #s 9944, 9283, 9931 and A718, respectively. Values are given as mean + S. D. The number of animals was 5, 5, 4 and 3 each for i.v. and i.g. studies for mice, rats, rabbits and monkeys, respectively. Results are shown in table below.

Table 21. **Percent DRSP elimination in urine and feces in mice, rats, rabbits and monkeys**

	Mice		Rats		Rabbits		Monkeys	
	Intravenous 5 mg/kg	intragastric 5 mg/kg	intravenous 4.8 mg/kg	intragastric 4.8 mg/kg	intravenous 0.5 mg/kg	intragastric 5 mg/kg	intravenous 0.5 mg/kg	intragastric 1 mg/kg
Urine	8.5 + 2.7	5.4 +2.9	9.2+1.6	13.7+4.1	42.8+2.8	40.7+3.8	23.5+6.1	19.7+2.9
Feces	70.6+5.2	66.8+4.1	89.4+7.7	75.0+5.4	48.2+3.6	52.1+5.1	56.6+3.4	61.2+4.4

Under the experimental conditions used, it was stated that excretion of ^{14}C-labelled compounds was almost complete after 24, 96, 72 hours in mice, rats and rabbits, respectively and within 15-20 days in monkeys.

Pharmacokinetic parameters of Yasmin in humans:

The table below contains the mean PK parameters of DRSP and EE following single and multiple dose administration of 3 mg DRSP/30 ug EE YASMIN tablets for comparison with PK data collected in various animal species.

Table 22. **PK of DRSP and EE in humans with DRSP/EE administration**

Mean (%CV) values

	Drospirenone					Ethinyl estradiol		
Cycle/day	Cmax (ng/ml)	Tmax (h)	AUC(0-24h) ng.h/ml	AUC ng.h/ml	T1/2 (h)	Cmax (pg/ml)	Tmax (h)	AUC (pg.h/ml)
1/1	36.5(29)	2.5(56)	308(23)	NA	NA	121.6(32)	2.2(34)	975(50)
1/21	59.5(32)	2.4(49)	754(34)	1502(36)	28.3(23)	145.7(40)	2.3(42)	1175(52)
3/1	39.6(30)	2.2(64)	376(33)	NA	NA	107.7(25)	2.4(42)	900(48)
3/21	60.4(33)	2.7(51)	814(33)	1675(36)	29.5(21)	143.3(26)	2.6(52)	1320(42)

Data thus showed that accumulation of drospirenone and EE with respect to AUC and Cmax within a treatment cycle. However no change in drospirenone Cmax and AUC values was seen between different treatment cycles.

Comment: It should be pointed out that under study No. A154, sponsor reported a mean AUC value of 260 ng.h/ml after oral administration of the anticipated dose of 2 mg DRSP in young women. However, under study No. A705 (P.5.12615) a value of 1589 ng.h/ml was reported with a dose of 3 mg DRSP + 30 ug EE/day.
It was not described if these variations were due to analytic methodology, different drug batches or different age population.

Protein binding of DSRP: Blank serum samples from humans and monkeys and plasma samples from rats and rabbits were spiked with DSRP to achieve concentrations of 50-5000 ng/ml. Additionally, about 0.14-0.19 ng/ml ^{3}H-DRSP were added to each sample. Following incubation for 1 hour at 37 C, free and protein-bound DRSP was separated by ultrafilteration.

Results: It was reported that in humans, monkey, rats and rabbits, about 95-97 % of DRSP was bound to proteins in serum and plasma. Binding was independent of the drug concentration.

Comments: The extent of DRSP protein binding was similar to that reported for other progestins.

Summary: DRSP Pharmacokinetic parameters after a single intragastric dose are compared for various species and humans in the following table:

Table 23. **Comparative PK of DRSP in mouse, rat, rabbit, monkey and human**

Species	Dose (mg/kg)	Cmax (ng/ml)	Tmax (h)	T1/2 (h)	AUC (ng.h/ml)	Bioavailability
Mouse	10	532	0.5	1.4	627	38
Rat	10	1440	2.0	3.0	8641	167
Rabbit	5	147	2.5	2.9	993	43
Monkey	10	1846	3.0	20	30367	55
Human	Single HTD	36	2.5	NA	NA	-
Human	Multiple HTD	60	2.4	28	754	-

Human dose was 3 mg DRSP/30 ug EE (i.e. 60 ug/0.6 ug/kg).

Although accumulation of drospirenone did occur with respect to Cmax and AUC within a treatment cycle, in another study treatment over 13 cycles demonstrated that serum Cmax, Tmax and AUC remained virtually unchanged.

TOXICOLOGY:

General comments: All acute, subchronic and chronic toxicity studies in rats and monkeys have been reviewed under the original IND submission dated 10-7-1996, a copy of which is appended. These studies are also summarized in this review.

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Addendum 1

Histopathology Inventory for MOUSE CARCINOGENICITY STUDY N-21-098

Study SGG001				
Species	Mice			
Adrena	•			
Aorta	•			
Bone Marrow smear				
Bone (femur)				
Brain	•			
Cecum	•			
Cervix				
Colon	•			
Duodenum	•			
Epididymis				
Esophagus				
Eye	•			
Fallopian tube				
Gall bladder	•			
Gross lesions				
Harderian gland	•			
Heart	•			
Hyphophysis				
Ileum	•			
Injection site				
Jejunum	•			
Kidneys	•			
Lachrymal gland	•			
Larynx				
Liver	•			
Lungs	•			
Lymph nodes, cervical				
Lymph nodes mandibular	•			
Lymph nodes, mesenteric	•			
Mammary Gland	•			
Nasal cavity				
Optic nerves				
Ovaries	•			
Pancreas	•			
Parathyroid				
Peripheral nerve				
Pharynx				
Pituitary	•			
Prostate				
Rectum	•			
Salivary gland	•			
Sciatic nerve	•			
Seminal vesicles				
Skeletal muscle	•			
Skin	•			
Spinal cord	•			
Spleen	•			
Sternum	•			
Stomach	•			
Testes				
Thymus	•			
Thyroid	•			
Tongue	•			
Trachea	•			
Urinary bladder	•			
Uterus	•			
Vagina	•			
Zymbal gland				

organ weight obtained

MOUSE CARCINOGENICITY STUDY

Study Title: Ethinyl estradiol (ZK 4944) and Drospirenone (ZK 30595) Oncogenicity study by oral gavage administration to female CD-1 mice for 104 Weeks.

Study Number: Schering (No. AZ86)
Volume Numbers: 1.15-1.19
Test Facility:
Study Date(s): Jan. 1996 to Feb. 1998
Date of Submission: May 14, 1999
GLP Compliance/Quality Assurance: Yes
QA Report- Yes (*) No ()
Study Type: Carcinogenicity
Species/strain: Mice/CD-1
Number of animals per group; age at start of study: 55 females/treatment group and 110 females in control group; 21-28 days of age at start.
Animal housing: housed individually
Drug Lot/Batch number(s): Following drug batches #s were used during the course of the study: Placebo batch #s: G/94059P-1 to G/94058P-5 (control)
ZK 4.944/Zk 30.595: G/94058B-1 to G/94058B-5(EE+DRSP combination)
ZK 4.944: G/94058A-1 to G/94058A-5(ethinyl estradiol alone)
ZK 30.595: G/94058C-1 to G/94058C-5(drospirenone alone)
Drug Purity/Stability/Homogeneity: The prepared vehicle was used within one week of preparation. All formulations were used within 6 hours of preparation. Formulations were homogeneous.
Doses: Because of the low dose levels administered, the test material was used as triturates in lactose. Ethinyl estradiol was used at 0.05% in lactose and drospirenone at 5.0% in lactose Same concentrations were used in combination. Placebo was lactose at a concentration of 20 mg/ml.

Table 1. **Study design for the mouse carcinogenicity study**

Group	Treatment	Dose level (mg/kg/day) EE	DRSP	Number of females Main study	Satellite study
1	Control	0	0	110	8
2	EE + DRSP	0.01	1.0	55	39
3	EE +DRSP	0.03	3.0	55	39
4	EE + DRSP	0.1	10.0	55	39
5	EE	0.01	0	55	23
6	EE	0.03	0	55	23
7	EE	1.0	0	55	23
8	DRSP	0	1.0	55	17
9	DRSP	0	3.0	55	17
10	DRSP	0	10.0	55	17

Basis of Dose Selection: Sponsor conducted a 14-week dose range-finding study (Report No. AB70) which was reviewed under the original IND submission. Ten treatment groups were used as detailed in table for group assignment for the carcinogenicity study. Animals in groups 2, 3 and 4 were gavaged orally with combination of EE/DRSP at dose levels of 0.03/3.0, 0.1/10.0 and 0.3/30.0 mg/kg/day. Animals in groups 5, 6 and 7 received EE alone and those in groups 8, 9 and 10 DRSP alone. These dose levels on body weight basis represented 50, 167 and 500 times, respectively the HTD of 3 mg DRSP + 0.3 mg EE/women/day. However, even though no treatment-related mortality was reported, sponsor decided to use the high dose of 10 mg/kg/day DRSP and 0.1 mg/kg/day for EE in the carcinogenicity study. Sponsor stated that this was decided based on previous experience with EE that doses greater than 0.1 mg/kg/day for EE will result in excessive mortality in the 2 years study period. To keep the ratio of DRSP/EE similar to that proposed for human use, the highest dose for DRSP was 10 mg/kg/day.

Relation to Clinical Use: The proposed clinical dose is 3 mg DRSP with 0.03 mg EE/day/50 kg women. The high dose of 10 mg DRSP/kg/day with 0.1 mg EE/kg/day, represents 167 times the HTD.

CAC Concurrence: None

Restriction Paradigm for Dietary Restriction Studies: None

Route of Administration: Oral gavage

Frequency of Drug Administration: once daily

Dual Controls Employed: No

Interim Sacrifices: None

e PK or Special Study Group(s): satellite groups for TK sacrificed at week 53.
...g week 53, blood samples were taken immediately before sacrifice from 4 and 3 mice in
ch treatment group for DRSP and EE determination. Similar number of mice were also bled
ter 30 minutes, 1, 2 and 4 hours after dosing.
ver samples were also collected during week 53 from satellite animals killed at the pre-dose
mpling occasion for DNA adducts analysis.
ascheduled Sacrifices or Deaths: yes

Deviations from original Study Protocol: Study was initially planned for 80 weeks
- but later it was decided to extend it to 104 weeks.
- **Study Results and Frequency of Monitoring:**

Clinical Observations: week 1- daily; week 2-4 twice weekly; weeks 5-13 once weekly and weeks 14 onwards once every 2 weeks. Animals were also examined pre-dose every day.

It was reported that there were no clinical signs related to treatment and no significant effect on the number of animals bearing palpable masses. The incidence of ungroomed coat was high when compared to control for females receiving the highest combination dose level and in those receiving EE at 0.1 mg/kg/day. At the low and intermediate dose level combinations or the intermediate dose level of EE alone, the incidence gradually increased as treatment progressed.

Mortality: checked every day. Animals in extremis were killed.
- As shown in **Table 2** below, the survival rate was significantly decreased during the 104- weeks
- treatment in high dose EE alone and to some extent in mid and high dose combination.

Group	1	2	3	4	5	6	7	8	9	10
Number of animals	110	55	55	55	55	55	55	55	55	55
Mortality	56	32	40	38	30	35	48	20	27	23
% Survival	49	42	27*	31*	45	36	13**	64	31	58

Palpable swellings: Shown in **table 3** below, compared to controls the number of animals with swellings and total number of swellings was higher in groups 6 and 7 (mid and high dose EE alone groups). The incidence was similar to controls for other groups.

Group #	# of animals with swelling	Total # of swellings
1	9	10
6	22	26
7	18	24

Body Weight: Each animal was weighed during the acclimatization period, on the day that treatment commenced, at weekly intervals for the first 14 weeks of treatment, once every 4 weeks thereafter and, then before necropsy.

Mean body weight at week 104 of study; body weight gain and body weight gain expressed as % of control is given in **table 4** below:

Group #	1	2	3	4	5	6	7	8	9	10
B.Wt.	35.9	36.9	37.7	36.6	36.4	38.7	41.1	36.9	37.5	38.2
0-104 wk wt.gain	14.8	16.1	16.8	15.8	15.2	17.8	20.2	15.9	17.2	17.2
Wt.gain as % of control		109	114	107	103	120	136	107	116	116

Initial body weight for mice at the start of the study ranged from 20.4 –21.4 g.

Food Consumption: recorded weekly for the first 14 weeks of treatment and once every 4-weeks thereafter. Food consumption was slightly higher for animals receiving EE at 0.03 and 0.1 mg/kg/day than the controls (114% for both treatment groups).
Water consumption: Water consumption was increased in mid and high dose combination groups and in mid and high dose EE alone groups. Water consumption was unaffected in females given DRSP alone and in low dose combination or low dose EE alone
Ophthalmoscopy: not performed
Hematology: Blood samples were taken during week 103 from surviving main study animals in groups 2, 4, 5, 8 and 9; 19 animals from groups 1 and 6; 18 animals from groups 3 and 9 animals in group 7. Samples were analyzed for PCV, Hb, RBC and WBC.
During week 53, blood samples were taken before sacrifice from satellite groups.

Hematology parameters determined during week 103, when compared to controls, demonstrated a treatment-related decrease in packed cell volumes, hemoglobin concentrations and erythrocyte counts as shown in **table 5** below (Mean + S.D):

Group	1	2	3	4	5	6	7	8	9	10
PVC	0.39 + 0.03	0.37 + 0.02	0.36 + 0.03^{a}	0.36 + 0.03^{a}	0.35 + 0.06^{b}	0.34 + 0.03^{c}	0.34 + 0.02^{b}	0.38 + 0.04^{b}	0.36 + 0.03	0.38 +0.04
Hb g/dl	12.5 + 1.0	12.3 + 0.8	11.7 + 1.0^{a}	11.7 + 1.0^{a}	11.5 + 1.9^{b}	11.3 + 1.0b	11.2 + 0.8b	12.4 + 1.3	11.7 + 1.0a	12.5 + 1.2
RBC10^{12}	8.40 + 0.78	7.68 + 0.56b	7.17 + 0.60c	7.05 + 0.68c	7.37 + 1.25c	7.07 + 0.63c	6.70 + 0.41c	8.42 + 0.98	7.83 + 0.66a	8.37 + 0.75

a - $p<0.05$ b - $p<0.01$ c - $p<0.001$ Compared to control group

- **Clinical Chemistry:** not performed
- **Organ Weights:** Compared to controls, as shown in **table 6** below, organ weight changes after 104 weeks of treatment consisted of decreased weight of uterus and thymus and increased weight of kidney and liver in animals which received EE alone or in combination with DRSP. Ovary weight was lower in females receiving 3 or 10 mg/kg/day DRSP. Values are relative to body weight (mean + S.D).

	1	2	3	4	5	6	7	8	9	10
B.wt (g)	35.1 + 3.8 (52)	34.8 + 4.7 (21)	35.1 + 4.1 (12)	3.5 + 4.8 (15)	33.1 + 3.6 (23)	36.6 + 4.8 (20)	39.1 + 3.5 (7)	35.0 + 3.1 (34)	36.3 + 4.9 (27)	36.7 + 4.9 (31)
Kidney	1.23+ 0.18	1.30+ 0.21	1.36+ 0.20	1.39+ 0.16^{b}	1.51+ 0.58^{a}	1.38+ 0.18^{b}	1.48+ 0.287	1.29 + 0.24	1.26 + 0.28	1.21 + 0.16
Liver	5.04 + 0.92	5.91 + 0.97^{b}	6.29 + 1.03^{b}	6.68 + 1.03^{b}	6.09 + 0.94^{b}	6.57 + 1.02^{b}	7.27 + 0.80^{b}	5.06 + 0.67	5.62 + 2.03	5.41 + 0.72
Thymus	0.195 + 0.407	0.122 + 0.117	0.066 + 0.026^{a}	0.067 + 0.038^{a}	0.110 + 0.094	0.076 + 0.030^{a}	0.054 + 0.025^{a}	0.160 + 0.244	0.271 + 0.575	0.128 + 0.064
Uterus + Cervix	3.28 + 4.28	1.38 + 1.41^{b}	0.81 + 0.30^{b}	1.01 + 0.97^{b}	1.27 + 0.47^{b}	1.41 + 1.82^{a}	1.16 + 0.91^{a}	3.01 + 2.94	1.62 + 2.12^{a}	0.84 + 0.76^{c}
Ovaries + oviducts	2.40 + 3.26	1.85 + 1.19^{a}	2.75 + 3.27	1,66 + 1.95	1.77 + 2.08	3.37 + 4.48	4.35 + 3.26	1.70 + 2.64	1.17 + 1.45^{a}	1.00+ 1.22^{b}

a= $p < 0.05$ b = $p < 0.01$ compared to group 1.

Gross Pathology: Gross pathologic changes are tabulated in **table 7** below for all animals.

Group #	1	2	3	4	5	6	7	8	9	10
# of mice	110	55	54	55	55	55	55	55	55	55
Brain,depression from pit.mass	1	5	12	16	3	22	39	0	1	0
L.N.madibular dark	3	4	5	8	6	10	10	4	2	2
L.N.mesenteric, dark	21	21	20	24	17	23	33	9	14	7
Mammary area, thickened	1	3	3	7	1	7	8	0	3	0
Pituitary, masses	5	12	19	25	10	27	47	1	3	2
Spleen, swollen	58	25	28	35	33	29	33	22	39	25
Thymus masses	23	11	5	4	14	3	5	11	16	20
Uterus, cystic	82	35	32	34	38	38	39	44	36	27
Uterus, distended	61	11	8	9	15	21	20	27	14	5
L.N. Axillary., mass	1	1	2	2	4	5	7	1	3	1
Gall bladder, distended	22	9	10	10	8	15	9	21	17	19
Harderian glands, masses	0	1	2	1	3	1	1	3	3	3
Skin masses	6	11	10	11	7	15	14	1	5	3
Stomach thickened wall	2	4	2	4	2	2	8	1	0	0
Urinary bladder, thickened wall	1	1	2	2	0	4	7	1	1	0

Thus significant finding when compared to controls present with EE or high dose combination treatment were: high incidence of pituitary masses, high incidence of dark lymph nodes, increased incidence of masses on the skin, higher incidence of thickened mammary area and thickened stomach and urinary bladder walls. There was low incidence of cystic and distended uterus with EE treatment. Low incidence of cystic and distended uterus was also observed with 10 mg/kg/day DRSP dose.

Histopathology:

Treatment-related neoplastic findings are tabulated in the following **table 8**:

Group	1	2	3	4	5	6	7	8	9	10
EE (mg/kg/day	0	0.01	0.03	0.1	0.01	0.03	0.1	0	0	0
DRSP (mg/kh\g/day)	0	1	3	10	0	0	0	1	3	10
# of mice examined	110	55	54	55	55	55	55	54	55	55
Pituitary adenoma	4	11[b]	13[c]	23[c]	7[a]	27[c]	41[c]	1	0	0
Pituitary carcinoma	0	0	3[a]	0	0	1	1	0	0	0
Uterine adenocarcinomas	0	0	6[b]	2	4[a]	10[c]	9[c]	0	1	0
Mammary glands Carcinomas	2	3	5[a]	2	0	6[a]	5[a]	0	1	1
Malignant adenocanthomas	1	3	3	2	2	5[a]	4[a]	1	1	0
Malignant carcinomas	0	0	2	0	1	1	2	0	0	0
Adenomas	0	1	1	0	0	0	0	0	0	0
Benign adenocanthomas	0	0	0	0	0	0	1	0	1	0
Benign fibroadenomas	0	0	0	0	1	0	0	0	0	0
All benign and malignant tumors	3	7[a]	11[c]	4	4	12[c]	12[c]	1	3	1
Uterine benign stromal polyps	14	13	11	6	7	17[b]	13	4	12	10
Malignant lymphoma	24	11	5	5	12	10	4	9	13	17
Harderian gland carcinoma	0	0	0	1	1	1	1	0	1	3

Significant increased in the incidence of uterine benign stromal polyps was observed only in group given mid dose EE alone and as such was considered probably not treatment related. a- p<0.05; b – p<0.01; c – p < 0.001

Neoplastic findings: A significant increase in pituitary adenomas and uterine adenocarcinomas was observed in groups given EE alone or in combination with DRSP when compared to controls. Mammary gland tumors in low and mid dose but not in high dose. Hardarian gland carcinoma in high dose DRSP.

Non-neoplastic findings: Treatment-related non-neoplastic findings were seen in a number of tissues, including uterus, uterine cervix, vagina, adrenals, femur, sternum, thymus, liver, mammary glands, pituitary, salivary glands and mesenteric and mandibular lymph nodes. The incidence of various findings is included in **table 9** below:

Group #	1	2	3	4	5	6	7	8	9	10
EE (mg/kg/day)	0	0.01	0.03	9.1	0.01	0.03	0.1	0	0	0
DRSP (mg/kg/day)	0	1	3	10	0	0	0	1	3	10
Number of animals examined	110	55	54	55	55	55	55	54	55	55
Uterine adenomyosis	9	7	35^{c}	16^{c}	14^{b}	46^{c}	46^{c}	2	7	6
Cystic endometrial hyperplasia	86	50	28^{c}	45	46	21^{c}	29^{b}	45	38	36
Uterine atrophy	10	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	1	1	4	13^{a}
Increased stromal collagen	0	0	1	2	0	1	5^{b}	0	0	0
Uterine cervix, increased stromal collagen	9	7	11^{a}	0^{a}	12^{a}	20^{c}	25^{c}	2	1	0
Vaginal epithelial atrophy/thinning	40	4^{c}	3^{c}	0^{c}	4^{c}	1^{c}	4^{c}	3^{c}	6^{c}	16
Mammary galnd acinar/ductular hyperplasia	25	29^{c}	19	29^{c}	28^{c}	37^{c}	36^{c}	14	13	8
Secretary activity	38	27	30^{a}	39^{c}	30^{a}	43^{c}	46^{c}	24	13	18
Ductular dilatation	33	27^{a}	27^{a}	36^{c}	26^{a}	43^{c}	43^{c}	23	11	18
Adrenal cortical pigmentary degeneration	16	35^{c}	45^{c}	48^{c}	34^{c}	45^{c}	51^{c}	10	1^{a}	5
Hypocellarity of zona fasiculata	2	3	6^{a}	8^{b}	0	1	7^{b}	0	0	0
Femur, osteosclerosis	5	3	7	12^{b}	2	7	23^{c}	0	1	1
Fibro-osseus proliferation	0	1	2	8^{c}	0	1	2	1	1	0
Sterum osteoscelerosis	2	2	5^{a}	10^{c}	2	5^{a}	11^{c}	1	1	0
Hepatic extramedullary hematopoiesis	26	11	17	13	13	24^{a}	23^{a}	3^{b}	4^{a}	8
Pigment in Kupffer cells	26	17	25^{b}	28^{c}	13	25^{b}	30^{c}	8	4^{a}	9
Salivary gland increased acinar/ductular eosinophila	0	0	19^{c}	14^{c}	0	13^{c}	16^{c}	0	0	0
Kidney										
Dilated cortical tubules	15	5	8	7	5	12^{a}	22c	2	6	4
Coticall cysts	3	1	7^{b}	7^{a}	2	5^{a}	5	0	1	2

Dilated Bowman spaces	14	2	5	10	5	9	12	2	4	7
Hydronephrosis	2	1	1	2	1	2	5[a]	0	0	2
Thymic involution	21	6	12	25[c]	4	7	20[a]	6	3	4
Lymphoid hyperplasia	34	7	3[b]	7[a]	6	6	7[a]	14	11	19
Mesenteric lymph nodes										
Erythrophagocytosis	19	17[a]	21[c]	27[c]	17[a]	21[c]	29[c]	11	9	8
Hemosiderosis	6	4	5	12[b]	6	5	11[b]	2	1	1
Manibular lymph nodes										
Erythrophagocytosis	3	3	4	8[b]	1	5[a]	6	1	0	1
Hemosiderosis	33	8	16	31[b]	14	24[a]	26[a]	7	5	11
Spleen										
Extramedullary hematopoiesis	56	32	39[a]	46[c]	29	41[b]	47[c]	19	30	20
White pulp hyperplasia	21	4	6	5	8	10	2[b]	13	15	14

a- $p<0.05$; b – $p < 0.01$ c – $p< 0.001$ All comparisons against control group.

Most significant treatment-related changes were observed in groups treated with ethinyl estradiol alone or ethinyl estradiol in combination with drospirenone. These changes included increased incidences of uterine adenomyosis, increased stromal collagen in the utrine cervix, higher incidences of acinar/ductular hyperplasia and ductular dilatation and secretory activity in mammary glands, increased adrenal cortical pigmentary degeneration and incidence of hypocellularity of the zona fasciculata, increased incidence of osteosclerosis of the femur and sternum, increased extramedullary hematopoiesis in liver, higher incidence of dilated cortical tubules and hydronephrosis in the kidney, involution of the thymus, and extramedullary hematopoiesis in the spleen. Spleen white pulp hyperplasia was significantly lower in high dose EE alone group.

The following significant non-neoplastic findings were stated to have uncertain relationship to treatment:

Lower incidence of mineralization of the brain in animals given EE
Higher incidence of angiectasis in the mesenteric lymph nodes of animals given low or high dose level of combination
Lower incidence of hemorrhage/thrombus in the ovaries of animals given low dose level of combination or 0.03 or 0.1 mg/kg/day EE
Acute keratitis in the eyes of animals given EE at 0.1 mg/kg/day either alone or combination, corneal ulcers in animals given EE alone at 0.1 mg/kg/day, acute iritis and pus in the anterior chamber in animals given the highest dose level of the combination and
lenticular degeneration in animals given DRSP alone at 10 mg/kg/day.

Toxicokinetics: After 104 weeks of treatment blood samples were obtained at pre-dose, 30 minutes, 1, 2, 4, 6 and 12 hours after dosing from 3 mice/g for EE and DRSP determination. For EE determination blood samples were collected in groups 1, 5, 6 and 7.
For DRSP determination blood was collected from all 10 treatment groups. At each sampling time freshly prepared 10% v/v 0.25M ———— was added immediately after collection to samples for DRSP analysis. As shown in **table 3 appended,** multiple of the human

exposure with high dose of DRSP was 2.9, which was similar to that observed at 53 week.

Overall Interpretation and Evaluation

Adequacy of the carcinogenicity studies and appropriateness of the test model: These studies were conducted by the sponsor without prior consultation with the Division. Study protocols were not submitted for prior concurrence regarding doses used. Dose selection was based on MTD (mouse) and multiples of human exposure (rat).

- The model used was appropriate and is routinely used for estrogen-progesterone combination treatment.
- **Evaluation of Tumor Findings:** The majority of the findings occurred in groups receiving ethinyl estradiol, either alone or in combination with drospirenone. These were attributed to pharmacological or exaggerated pharmacological activity of EE. By contrast, there were no significant treatment-related neoplastic findings in animals given DRSP alone except for an increased incidence of Harderian gland carcinomas.
- In the rat carcinogenicity study only neoplastic finding with DRSP alone was the increased incidence of adrenal gland benign pheochromocytoma as well as benign + malignant pheochromocytoma in the high dose animals.

Summary: Conclusions and Recommendation

- Acceptability of Study(s) or Overall Testing Approach: Study is acceptable. Overall testing approach was similar to that used for other estrogen-progestin-combined oral contraceptives.
- **Major Tumor Findings:** There was a statistically significant positive-dose relationship in ituitary adenomas among the combination and ethinyl estradiol alone groups. There were statistically gnificant positive-dose relationships in carcinomas and carcinosarcomas of the mammary gland, and of pituitary adenomas among groups treated with ethinyl estradiol alone.
- There was a positive-dose relationship of carcinomas in harderian glands with drospirenone alone.

- **Non-neoplastic Findings:** as given in table 9 on page .
- **Biological Significance:** Observed findings are similar to those observed with other estrogen progestin combination oral contraceptives except for pheochromocytomas observed in DRSP treated rats.
- **Potential Clinical Implications of Findings:** Essentially same as with other E-P combined oral contraceptives.
- **Recommendations for Further Analysis:** None
- **Rat carcinogenicity study:** Reviewed under original IND submission
- **Note:** Both the mouse and rat carcinogenicity studies were presented to Exec-CAC on 1-4-2000 (appended).
- **Executive committee recommendations and conclusions:** The committee felt that the mouse carcinogenicity study was acceptable, based on increased mortality in the mid and high dose combination and high dose EE alone groups and that the tumors observed should be reported.

- The rat study was considered acceptable if the AUC data (which demonstrate a 10 fold higher exposure in the HD animals compared to humans) are valid. There are questions as to how much of the AUC was measured versus how much was calculated from extrapolation. The information provided ggests that a large fraction (over half) of the AUC was contributed by extrapolation, which would not

port the value used for the $AUC_{0-24}h$.
The sponsor was asked to provide evidence that the extrapolation from $AUC_{0-4}h$ to $AUC_{0-24}h$ is valid.

IMMUNOTOXICOLOGY: None submitted

REPRODUCTIVE TOXICOLOGY: Reproductive toxicology studies have been reviewed under the original IND submission dated 10-7-1996. Copy of the IND review dated 10-17-1997 is appended and results also have been summarized under present NDA summary.

GENETIC TOXICOLOGY: Reports on Ames test, HGPRT-test with V79 cells, clastogenic potential in the human lymphocyte test, unscheduled DNA synthesis, mouse micronucleus test and DNA adduct analysis with liver of treated male and female rats were reviewed under original IND submission dated 10-7-1996. Copy of the original IND review is appended and results have also been summarized under NDA summary.

In addition, under I ——— Annual report dated 9-27-1999, sponsor has provided summary on DNA-adduct levels in liver of female CD-1 mice killed 53 weeks after daily DRSP + EE administration during the 2-year mouse carcinogenicity study **(Report No. AW44).**
Also under **Study No. AS78,** summary results of a study entitled "DNA-adduct analysis in female rat hepatocytes after incubation with ^{3}H-labeled compound were provided.

<u>Results</u> **(study No. AW44):**

<u>Drospirenone-</u> No DNA adducts was detected in the 1 and 3 mg/kg DRSP treated groups. With high dose of 10 mg/kg DRSP, three DNA adducts were detected and the calculated relative adduct labeling (RAL) values were 40 and 57 adducts/10^9 nucleotides

<u>Ethinyl estradiol-</u> No DNA adducts detected at any dose level

<u>DRSP + EE-</u> With low and mid dose levels no DNA adducts were detected. With the high dose combination 3 DNA adducts were detected. RAL values were calculated as 11 and 12 adducts/10^9 nucleotides.

<u>Results</u> **(study No. AS78):** No tritium-labeled DNA adducted were generated in rat hepatocytes in vitro.

Results of a study entitled "DNA repair synthesis in hepatocyte primary cultures from rats and humans of both genders exposed to drospirenone" were provided under **study No B839.**

From the results of this study it was concluded that drospirenone at concentrations ranging from 1-50 umol/l, elicited DNA-repair synthesis in primary hepatocytes from rats of both genders, but was inactive in primary hepatocytes from human donors of both genders. Each experiment included negative and positive controls.

SPECIAL TOXICOLOGY STUDIES: None reported.

OVERALL SUMMARY AND EVALUATION:

Introduction: Drospirenone is a derivative of 17-a-spironolactone. It has progestational, aldosterone-antagonistic and anti-androgenic properties.

ADME

After oral administration, DRSP is rapidly absorbed, bioavailability being dose-dependent and approached 100% at high doses in rats and mice. Cmax and AUC increased with dose in mice, rats and rabbits. Multiple dosing did not suggest systemic accumulation. DRSP is rapidly eliminated in the mouse, rat and rabbit because of its short $T_{1/2}$ and increased total metabolic clearance. Elimination was complete by 24, 72 and 96 hours in the mouse, rabbit and rat respectively. In the monkey it took 15-20 days, total metabolic clearance being about 25% of the plasma flow rate to the liver. Excretion was primarily in the feces in the rat, mouse and monkey and equal in feces and uterine in rabbits. Major human metabolites M 11 and M14 were identified in plasma of rats, mice, rabbits and monkeys. M14 was minimal in rats.

General Pharmacology: Drospirenone's contraceptive action is attributed to its suppression of gonadotropins. Drospirenone has been shown to have progestogenic, anti-mineralocorticoid and antiandrogenic activities. It exerted no detectable androgenic, estrogenic or glucocorticoid activities. Drospirenone isomerizes in acidic pH to ZK 35096, which was reported to have no hormonal activity.

Safety Pharmacology: Drospirenone did not prolong hexobarital-sleeping time in mice. In combination with EE, it resulted in a dose-dependent prolongation of the CNS depressant action of hexobarbital. Drospirenone had a short lived neurotoxic effect in mice. It had no effect on arterial BP and heart rate or vascular activity. Drospirenone decreased pulmonary intraplural resistance in rabbit but had no effect on intestinal motility and intraluminal pressure.

Toxicity Evaluation: The toxicological profile of DRSP, EE or combination of the two (100:1 or 10:1) were evaluated in female animals in acute, single and repeat dose systemic tolerance, carcinogenicity, reproductive studies and genetic toxicology studies.

In dogs after a single oral dose of 250 mg/kg, plasma fibrinogen was increased in all animals on days 2, 4 and 8. Also serum free Hb was increased. No further studies were conducted using dogs.

Daily administration of DRSP by gavage in rats for 7 days at dose of 10, 50 and 100 mg/kg caused induction of N-demethylase activity at all dose levels. Also, microsomal P-450 content was increased with 50 and 100 mg/kg doses.

In a 14-week study in rats with EE/DRSP doses of 0.01/1.0, 0.03/3.0 and 0.1/10.0 mg/kg/day, enzyme induction was seen at all dose levels and increased P-450 at mid and high dose levels. Liver weight was increased. A significant decrease in Hb and MCHC was observed in both the combination and EE alone groups. Increased urinary sodium excretion was attributed to DRSP anti-mineralocorticoid activity. Organ weight changes and microscopic findings were consistent ɔnal effect of EE and DRSP. Body weight was increased significantly at all doses of ɪe.

ƽP alone at a high dose of 15 mg/kg/day in a 27-week toxicity study in rats, similar ere obtained as in the 14-week toxicity study.

ᴡeek rat toxicity study, changes observed in hematological and biochemical parameters as enzyme induction and organ weight changes were similar to those observed in the 14 week toxicity studies.

'-week DRSP toxicity study in monkeys, hematological and urinalysis changes were r to those seen in rats. Thrombin time was decreased and fibrinogen values were increased. ment decreased the relative weights of the liver, kidney and pituitary and increased that of ɪals. Progestogenic effects were seen in the uterus, ovaries and mammary gland.

ɪe 53-week EE/DRSP toxicity study in monkeys, glucouria and increased urinary K excretion ɪe high dose groups was reported. High dose combination caused enzyme induction and SP increased and EE decreased cytochrome. P-450. Liver weight was increased as in the rats. ɪer expected hormonal effects were observed.

the rat carcinogenicity study, systemic exposure of DRSP was about 12 fold higher than in ɪmans with therapeutic dose. Significant DRSP-related changes were an increase in body ɪeight and hematological changes as seen in other rat toxicity studies. Non-neoplastic ɪistopathological changes consisted of adrenal gland zona granulosa hyperplasia and cervix and ɪaginal epithelial atrophy. Significant neoplastic findings were an increased incidence of adrenal gland pheochromocytoma as well as benign + malignant pheochromocytoma.

In the mouse carcingenicity study, most significant treatment-related neoplastic changes consisted of an increased incidence of pituitary adenoma, uterine adenocarcinoma, and mammary gland benign and malignant tumors in EE alone or EE in combination with DRSP groups. Harderian gland carcinoma incidence was increased in DRSP high dose group.

In the genotoxicity testing, DRSP was negative in the Ames test, HGPRT-test with V79 cells, in clastogenic potential in the human lymphocyte test and in the mouse micronucleus test. DRSP did form DNA adduct in liver. However, using various steroids, it was shown that there was no correlation between the DNA adducts formation and tumorigenic potential. While DNA adducts were observed in the livers of mice given high dose of DRSP, these were not observed with EE alone administration.

In two experiments using identical procedures, a positive response observed in the unscheduled DNA synthesis was reproducible and dose-dependent and not related to cytotoxic effects. Based on these findings, the sponsor concluded that DRSP possesses a genotoxic potential in primary hepatocytes in vitro. However, using negative and positive controls, it was shown that this did not occur with primary hepatocytes from human donors.

Clinical Relevance of Safety Issues: Drospirenone is a derivative of 17-a-spironolactone. Spironolactone has been shown to be a tumorigen in chronic toxicity studies performed in rats, with its proliferative effects manifested on endocrine organs and the liver. In a study using 25, 75 and 250 times the usual daily human dose (2 mg/kg) there was a statistically significant dose-related increase in benign adenomas of the thyroid and testes. In the female rats there was a statistically significant increase in malignant mammary tumors at the mid dose only. In the male rat a dose of 500 mg/kg produced hepatocytomegaly, hyperplastic nodules and hepatocellular carcinoma, the last was not statistically significant (PDR 51, 1997). Tumors similar to those produced by spironolactone were not seen in the DRSP rat carcinogenicity study. However, negative finding with DRSP could be due to the fact that high dose of DRSP was limited by the toxicity of EE.

Sponsor however, has pointed out that there are significant biotransformation differences between drospirenone and spironolactr which the differences in tumorigenic response between spironolactone and drospiren . Eighteen percent of an in vivo dose of spirnonolactone was metabolized to 6B, /B-epoxy canrenone, which is further reduced at position 3 to yield the 3a or 3B hydroxy derivatives of 6B,7B epoxy canrenone. These hydroxylated metabolites were shown to be direct acting mutagens in the mouse lymphoma assay.
In the case of drospirenone, the formation of 6B,7B epoxy metabolites is highly unlikely, since drospirenone contains a 6B,7B methylene bridge, which is not subject to epoxidation like the 6,7 double bond in canrenone.
Therefore, the formation of mutagenic drospirenone metabolites similar to those generated from canrenone is not expected based on the chemical properties of drospirenone.

Other Clinically Relevant Issues: In the rat carcinogenicity study, drospirinone increased the incidence of benign adrenal pheochromocytomas. This effect has not been seen with other contraceptive steroids. The relevance of this effect to humans is not known but only benign and not malignant pheochromocytomas were increased and a similar result was not seen in the mouse carcinogenicity study.

Observations made in the pre-clinical toxicity studies, which may be relevant clinically, were treatment-related effects on the adrenals and cytochrome P-450 dependent enzyme induction. However, as stated in the medical review, electrolyte monitoring in clinical studies did not indicate any effect of treatment on adrenal and kidney function. Also no enzyme induction was observed in humans. Whether observations of concern in rats is species specific or due to higher systemic exposure over longer time period is unknown. However, the effect of Yasmin in renal impaired humans has not been reported and is under investigation.

Conclusions: Pharmacology considers that the proposed ethinyl estradiol + drospirenone treatment as a combined oral contraceptive is safe based on preclinical findings and available clinical data.

Labeling Review (NDA): Sponsor has been requested to formulate labeling as for a new molecular entity (unlike other steroid progestogens), since Drospirenone is distinctly different from the presently marketed progestogens. Carcinogenesis, Mutagenesis and Impairment of

Fertility as well as Reproductive toxicity sections of labeling should fully describe the findings and the doses in these studies should be expressed as multiples of the human exposure (AUC).

RECOMMENDATIONS:

Internal comments: Rat and mouse carcinogenicity studies were presented to the Exec-CAC on 1-4-2000. The committee recommendations and conclusions are appended.

External Recommendations (to sponsor): Sponsor should modify the labeling as indicated in the Draft letter content for sponsor. Exec-CAC recommendations have been communicated to the sponsor.

Draft letter Content for Sponsor:

Future development or NDA issues: None

Reviewer signature/team leader signature [Concurrence/Non-concurrence]

cc: list /S/
Draft date (# of drafts):
Memorandum of Non-concurrence (if appropriate, attached): /S/
Addendum to review (if necessary):
Appendix/attachments: 1. Pharmacology review of the original IND submission dated 10-7-1996.
2. Comparative PK parameters in mice, rats and humans
3. Carcinogenicity studies presented to Exec.CAC
4. Exec.CAC comments and recommendations.

APPEARS THIS WAY
ON ORIGINAL

NDA 21-098

Attachment 1 to NDA review: reviews of pharmacology, repeat dose toxicity, reproduction and mutagenicity studies.

APPEARS THIS WAY
ON ORIGINAL

The pharmacologic profile of DRSP was characterized using in-vivo and in-vitro models of endocrinological (progestational, antiandrogenic/androgenic, antimeralocorticoid/minerolcorticoid, antiglucocorticoid/glucocorticoid and estrogenic) activity, vascular responsiveness and in-vitro biochemical assays to study receptor binding.

Progestational activity: DRSP exhibited progestational activity as it 1) promoted the maintenance of pregnancy in mice and rats, 2) inhibited ovulation in mice and rats, 3) promoted uterine endometrial transformation in rabbits and 4) exhibited high affinity for the progesterone receptor which was not affected by the presence of EE. In rat the concurrent administration of EE enhanced the progestational activity.

Antiandrogenic activity: 1) Depending on the route of administration, the antiandrogenic activi' of DRSP in male rats was found to be 3-9 times lower than that cyproterone acetate and equal to or about 3 times higher than that of spironolactone, 2) Given orally, DRSP decreased both serum LH and testosterone levels in male monkeys, the activity being 20-40 times greater than spironolactone, 3) Administration of 10 mg/animal/day of DRSP or 30 mg/animal/day of spironolactone to pregnant rats during the final trimester by oral or sc route resulted in feminization of male fetuses as judged by shortening of the anogenital distance and the length of urethra, and 4) in-vitro, the antiandrogenic activity of DRSP in the CAT-transactivation assay was determined to be 1/3 of cyproterone acetate.

Antimineralocorticoid activity: 1) of DRSP administered by either oral or sc route in rats and characterized by increased sodium excretion and an increase in urinary Na/K ratio, was 8 to 19 times greater than that of spironolactone and was not affected by administration of EE. It was stated that the physiological counter regulation i.e., increased serum aldosterone levels, in the rat does not overcome the Antimineralocorticoid activity of DRSP but does overcome the antialdosterone effect of spironolactone, 2) DRSP inhibited in vitro aldosterone-stimulated electrogenic sodium transport 10 times more effectively than either spironolactone or progesterone, and 3) In-vitro DRSP binds with high affinity to mineralocorticoid receptor. It does not exhibit any in-vivo mineralocorticoid activity.

DRSP did not exhibit any significant androgenic, estrogenic or glucocorticoid activity. In vitro it binds with low affinity to the androgenic and glucocorticoid receptors. It does not bind to estrogen receptor and does not interfere with EE binding to its receptor. It does not bind to cortisol binding globulin and has low affinity for steroid hormone binding globulin.

DRSP had no significant effect on vascular system. It did not affect smooth muscle tone in-vitro.

ADME for Drospirenone:

Following oral administration, absorption was rapid and almost complete in mice, rats, rabbits and monkeys.

Comparative PK of DRSP for mouse, rat, rabbit, and monkey after a single 10 mg/kg oral dose and in human after 3 cycles of therapeutic dosing (3 mg DRSP + 0.03 mg EE/day), is shown in table below:

Parameter	Mouse	Rat	Rabbit	Monkey	Human
Cmax(ng/ml)	532	727	294	1846	60
Tmax(hr)	0.5	1.22	2.5	3.0	2.5
T1/2(hr)	1.4	2.3	2.9	19.6	29.0
AUC (ng.h/ml)	627 0-∞	8641 0-∞	1986 0-96	21230 0-∞	784 0-24 1588 0-∞
MRT(hr)	1.7	5.3	7.2	28.3	40.0
Rel.bioavailability(%)	100	100	43	55	76
Cl(ml/min/kg	101	22.3	35.0	3.3	
Vss L/kg	1.72	5.9	10.5	2.4	
Multiple of HTD exposur	<1	5.4	1.3	13.4	

Metabolic clearance and volume of distribution are after i.v. dose of 1 mg/kg in rats and mice and 0.5 mg/kg in rabbits and monkeys. In monkeys bioavailability was lower after 10 mg/kg dose than after 1 mg/kg dose and unchanged drug was eliminated at a faster rate than 14C indicating the existence of metabolites with longer T1/2 than DRSP. The Cmax and AUC increased in mice, rats and rabbits with increasing dose.
In pregnant rats following both oral or iv administration of labeled DRSP, radioactivity was distributed throughout the body within 6 hours with high levels detected in liver, stomach, small intestines, adrenal glands, lacrimal glands, fat and eyes. High volume of distribution was also reported for mice and rabbits.

Regardless of route of administration, levels in the small and/or large intestine remained high at 24 hour but were eliminated within 48 hours.
In pregnant rabbits, highest radioactivity was observed in liver 15-30 minutes after dosing. In both rats and rabbits, radioactivity measured in fetuses was low and non detectable 24 hours post-dosing.

With short MRT and T1/2 and high metabolic clearance, elimination was essentially complete within 24 hours in mouse, 72 hours in the rabbit and 96 hours in the rat but in the monkeys it tool 15 to 20 days after a single oral or iv dose of labeled DRSP.

Excretion was primarily in the feces of rats (75-90%), mice (65%) and monkeys (57-61%). In rabbits it was 50% in feces and 40% in urine. Total metabolic clearance rates were close to or exceeded the plasma flow rate to the liver for mouse, rat and rabbit, indicating contribution of extrahepatic metabolism. In the monkeys total metabolic clearance rate was about 25% of the plasma flow rate to the liver.

ADME for ethinyl estradiol:

The bioavailability of EE was 5-7% in mice and 10-14% in rats and Cmax was observed 0.5 to 1.0 hour after dosing suggesting considerable first pass metabolism. Relatively short T1/2 of 5.6 to 8.6 hours in rats indicated rapid metabolism. In rats and mice MRT after oral dose was 4.4 to 10 hours and after iv administration was 0.5 hour in mice and 1.5 hours in rats, indicating rapid elimination. Vd in rats was 66.5 L/kg after oral administration suggesting wide distribution with a total plasma clearance of 73.9 ml/min/kg.
The absorption of EE increased linearly and exhibited dose proportionality after oral administration when given in combination with DRSP to cynomolgus monkeys.

Toxicity studies:

The toxicologic profiles of drospirenone, EE or combination of the two (100:1 or 10:1) were evaluated in female animals in acute, single and repeat dose systemic tolerance, carcinogenicity, reproduction studies and in genetic toxicology studies.
All toxicity studies subject to GLP regulations contained GLP compliance and QA statements.

Single dose lethality: LD50 for mice and rats when given DRSP by gavage was 1250 and by ip 500 and 250 mg/kg in mice and rats respectively. Deaths occurred within 3 to 4 days.

Clinical signs included apathy, gait and posture disturbances. At highest dose of 2500 mg/kg, twitches, spasms and/or jumping on stimulation were reported. NOEL after oral dosing was 500 mg/kg in mice.
Postmortem findings included pale liver and kidneys, yellow mucoid contents of small intestines or stomach, black covering of stomach after oral administration and white substance, most likely the drug in abdominal cavity after ip administration.

In dogs a single oral dose of 250 mg/kg or an iv dose of 0.165 mg/kg was well tolerated. Treatment-related effects included vomiting 5 hour post dosing, transient decrease in food and water consumption, slight increase in serum glucose, total protein, cholesterol, urea nitrogen, Na, Ca and chloride. Plasma fibrinogen was increased in all animals on day 2, 4 and 8. No animals died during the study and LD50 was not identified. With iv administration free Hb in serum was increased ($p<0.05$). Hb values for the NaCl vehicle, propylene glycol vehicle and test substance on day 1 after one hour were 28.2, 65.8 and 124.2 mg/dl respectively. No other changes were reported and no animal died.

Dose range finding study in female mice after daily intra gastric administration of either the compound combination or ethinyl estradiol or drospirenone alone over a period of 14-15 weeks. Report No. AB70.

This study was conducted to choose appropriate dose-levels for the mouse carcinogenicity study which is now ongoing.

Three groups of 30 female HAN:NMRI Schering mice (groups 2, 3 and 4) were gavaged intragastrically daily for 14 weeks with combination of EE/DRSP at dose levels of 0.03/3.0, 0.1/10 and 0.3/30 mg/kg body weight. Three groups of 25 mice each received EE alone (groups 5, 6 and 7) or DRSP alone (groups 8, 9 and 10) at doses used in the combination. 25 mice in group 1 were treated with vehicle alone (20 ml/kg). These dose levels on body weight basis represented 50, 167 and 500 times the human dose of 3 mg DRSP + 0.03 mg EE/woman/day.

Results:

Mortality and general observations: 15 mice died during the course of the study but none considered treatment-related. No dose-dependent treatment-related changes were observed.

Food and water consumption: was slightly increased.

Body weight: treatment resulted in increased body weight gain. % gain for groups 2-10 was 25.9, 31.0, 24.1, 29.3, 37.9, 34.5, 20.7, 19.0, and 20.7% respectively.

Hematologic changes: Changes seen in combination treatment groups and in EE alone were a decrease in RBC counts and an increase in MCH and MCV. Other changes included a decrease in leucocyte, lymphocyte and eosinophil counts. Platelet count was increased in low dose combination and in low and mid dose EE alone groups.

Organ weight changes: significant organ weight changes consisted of an increase in absolute and relative weights of liver, spleen, pituitary, pancreas, mandibular salivary glands and uterus. Absolute wt of kidney was also increased. Adrenal wt was decreased only with high dose DRSP alone. The weight of the ovaries was decreased. All these changes were attributed to pharmacologic activity of EE as these occurred both in the combination and the EE alone treatment groups.

Histopathological changes: significant treatment-related changes consisted of an increased incidence of basophilic condensation in the liver, which was suggested to be due to increased amount of RER indicating increased protein synthesis. There was increased incidence of brown atrophy and spindle cell hyperplasia in adrenal glands. In ovaries there was increased incidence of atrophy of interstitial glands and decreased luteal mass with suppression of sexual cycles. In uterus the incidence of hyperplasia/hypertrophy, glandular dilatation and fibrosis of the lamina propria was increased and there was increased incidence of diestrus. The sponsor suggested that this stimulatory effect on uterus may lead to tumorigenic responses on prolonged treatment and also form the basis for inflammatory diseases such as pyometra which could decrease survival rate. The cervix and vagina also showed an increased incidence of epithelial hyperplasia and cornification. There was an increased incidence of proliferation of the mammary glands, suggested as a possible sign of tumorigenic response. Increased hematopoiesis and megakaryocytosis was noted in the spleen. There was a depression in myelopoiesis which was suggested to be a consequence of increased osteosclerosis in sternum and femur. With regards to atrophic changes in ovaries, DRSP and EE had synergistic action but the stimulatory effect of EE on the uterus was antagonized by DRSP. Most of the above described changes were seen in the combination and EE alone groups.

Pharmacokinetics: Blood samples were collected at 0, 0.5, 1 and 4 hour for 5 mice/time point. ——— was added to blood to prevent degradation of DRSP. PK reported separately as AG46.

PK parameters for DRSP:

DRSP+EE mg/kg/day	Cmax ng/ml	Tmax Hour	AUC 0-4h ng.h/ml	AUC 0-24h ng.h/ml	AUC0-24h/Tx AUC0-24h/H
3.0 + 0.03	53	0.5	66	84	0.11
10 + 0.1	501	0.5	434	513	0.65
30 + 0.3	2460	0.5	2684	3734	4.76
3	108	0.5	116	117	0.15
10	718	0.5	1204	1316	1.68
30	4110	0.5	7862	9711	12.4

PK parameters of EE:

0.03	97	0.5	191	313	0.25
0.1	239	0.5	398	652	0.52
0.3	303	0.5	663	1176	0.94

AUC 0-24h was extrapolated from AUC 0-4 h for comparison with human AUC 0-24h. Multiple of human exposure was calculated on the basis of AUC 0-24h of 784 ng.h/ml for women on therapeutic dose of 3 mg DRSP + 0.03 mg EE/day.
PK data thus showed that at corresponding DRSP dose levels, the maximum concentrations were always higher after administration of DRSP alone than after administration of the DRSP/EE combination. Also after administration of an EE dose 500 time greater than the proposed human contraceptive dose, the systemic exposure achieved in mice was below that observed in human. Thus with respect to EE, pharmacodynamic parameters are better for dose selection for carcinogenicity study. However, the exposure to DRSP when given with EE was well below 10X the human exposure Pharmacology requires for carcinogenicity studies with steroids. It is however, possible that in the 2 year carcinogenicity study, exposure may increase with time.
Considering the observed sensitivity of mouse to steroid, Pharmacology considers that the doses selected are adequate for 2 year mice carcinogenicity study for DRSP/EE combination as long as high dose DRSP alone groups are also included.

Note: In the ongoing mouse carcinogenicity study maximum dose of DRSP/EE used is 10/0.1 and it may not be adequate.

Effect of drospirenone (ZK30.595) on liver growth and cytochrome P-450 dependent monooxygenase in liver microsomes after daily i.g. application over a period of 7 days in female rats. No.A861

Six Han:Wist Schering female rats/g were gavaged orally with dose of 10, 50 and 100 mg DRSP/kg. Control group received vehicle (10 ml/kg) which contained NaCl, Klucel LF, Myrj 53 and water.

Results: A dose-dependent induction of N-demethylase activity was observed from the low dose onward using ethylmorphine and benzphetamine as substrates. These activities were associated with increases in absolute and relative liver weights, DNA and protein content/total liver. Also increases in microsomal cytochrome P-450 content was observed from mid dose onward and was not dose-dependent.
It was not stated if similar enzyme induction occurs with any other marketed progestin and this reviewer could not locate any reference for such an effect.

Ethinyl estradiol plus drospirenone: systemic tolerance and dose range finding study in female rats after daily per OS (intra gastric) administration of either the compound combination or ethinyl estradiol or drospirenone alone over a period of about 14 weeks. Report NO. 9677

This study was conducted to determine appropriate dose levels to be used in the rat carcinogenicity study.

Ten groups of 20 female Han:WIST Schering rats were treated daily intragastrically by gavage for 14 weeks with either a combination of EE/DRSP at doses of 0.01/1.0, 0.03/3.0 and 0.1/10.0 mg/kg (groups 2, 3 and 4) or with each of the compound alone at the same doses (EE groups 5, 6 and 7) and DRSP (groups 8, 9 and 10) or with an equivalent volume of vehicle (control group 1).

Results:

Mortality: A total of 6 rats died during the course of the study from various groups with no treatment association.

General observations: there were no significant treatment-related effects.

Food and water consumption: food consumption was decreased in groups 6 and 7 and increased in group 10. There was no effect on water intake.

Body weight: EE alone at the mid and high doses respectively caused 15 and 36% decrease in body weight gain. DRSP alone increased body weight gain in a dose-dependent manner, values being 32%, 56% and 76% for the low, mid and high dose groups. Body weight gain during week 1-13 for the 10 groups was 55, 56, 61, 60, 47, 47, 35, 73, 86 and 97 g respectively.

Ophthalmologic examination revealed no treatment effects.

Hematology: decreases in RBC, Hb and MCHC were reported at week 5 for mid and high dose combination and/or EE alone groups.

Urinalysis: Only significant effect was an increase in the excretion of urinary sodium at the high dose combination or high dose DSRP alone. This was attributed to DRSP's aldosterone-antagonistic action. Values (umol/24h) at weeks 13 for groups 1 through 10 were 488, 504, 609, 857,483, 711,502, 453,622 and 730 respectively.

Biochemistry: EE administered alone or in combination with DRSP resulted in an increase in alkaline phosphatase and a decrease in serum cholesterol and chloride. Serum glucose was increased in groups given combination or either compound alone, effect was more consistent for DRSP alone groups. Urea nitrogen was increased in high combination group.

Blood coagulation: a slight but significant shortening of activated partial thromboplastin time (APTT) in groups 5 and 8, 9 and 10 in week 4 but not in week 13 was not regarded compound related.

Biochemical determination in liver microsomes and liver tissue:

A significant induction of N-demethylase activities using aminopyrine and benzphetamine as substrates were observed in all treated groups and with ethylmorphine in groups 4 and 7. Significant increase in cytochrome P-450 in liver microsomes was observed only in DRSP-treated animals in the mid and high dose groups.

Necropsy: only significant findings noted at necropsy were ovaries diminished in size in 1, 3 and 1 animals in mid, high dose combination groups and in high EE alone group.

Organ weights:EE alone or in combination with DRSP caused a sig increase in the absolute and relative weight of liver and

adrenals from mid dose onwards. High dose combination or EE alone decreased absolute and relative ovarian weight. Absolute and relative uterus weight was decreased with all combinations and with mid and high dose DRSP.
Absolute and relative weight of pancreas was increased in high dose combination group.

Treatment related microscopic findings: Eosinophilic hepatocellular changes in the liver in groups 3,4, 6 and 7 were considered signs of metabolic activation, lipid depletion in adrenals in these groups was considered related to hormonal activation of the cortical zone and ovarian changes (atretic follicles) in these groups were signs of antiovulatory effects. Hepatocellular hypertrophy was seen in groups 6, 7 and 10 was suspected to be treatment related. Vaginal cornification was decreased and mucification increased in high dose combination group.

MTD was not reached. Doses for the 2 year carcinogenicity study must be based on 10 times the human systemic exposure with therapeutic dose.

Ethinyl estradiol and drospirenone: Tumorigenicity study over 106 to 110 weeks in female rats with intra gastric administration. Report NO. AG63.

Ten groups of female rats (Han:Wist) were treated daily by oral gavage for a period of over 2 years with ethinyl estradiol, drospirenone or a combination of both. There were 50 rats each in groups 2-10 and 110 rats in control group 1 which were administered vehicle at a volume of 10 ml/kg.

Dose levels used were based on the results of the 14 week dose-range finding study.
Rats in groups 2, 3 and 4 were gavaged EE/DRSP at doses of 0.003/0.3, 0.03/3.0 and 0.1/10.0 mg/kg. Those in groups 5, 6 and 7 received an equivalent dose of EE and those in group 8, 9 and 10 DRSP alone.

During study weeks 105-107, plasma concentrations of EE and DRSP were determined in samples containing a specific inhibitor to prevent ex-vivo metabolism of DRSP. Results as shown in table below suggest that dose selection was appropriate.

AUC(0-24h), the ratio of rat/human of the AUC and maximum plasma concentration after ethinyl estradiol and/or drospirenone.

Group#	AUC(0-24h) EE	AUC(0-24h) DRSP	EE-ratio AUC(0-24h)rat/ AUC(0-24h)human	DRSP-ratio AUC(0-24h)rat/ AUC(0-24h)/human	Cmax EE	Cmax DRSP
2	n.d	760	n.d	1.0	n.d	228
3	n.d	2983	n.d	3.8	n.d	370
4	n.d	7962	n.d	10.2	n.d	850
5	n.d	-	n.d	-	n.d	-
6	0.385	-	0.3	-	0.041	-
7	1.377	-	1.1	-	0.243	-
8	-	472	-	0.6	-	122
9	-	3154	-	4.0	-	482
10	-	9644	-	12.3	-	1200

n.d.+not determinable. Human AUC (0-24h) for EE and DRSP (0.03 + 3.0) were 1.2 ng.h/ml and 784 ng.h/ml respectively from Schering report NO.AG470.

Mortality: The morality rate (%) in control and 9 treated groups was 41, 38, 35, 31, 36, 36, 47, 27, 44 and 53% respectively. Major cause of death was pituitary adenoma which was highest in the control group, uterus adenocarcinoma (which was dose-related in EE groups) and gavage errors. Other causes which were randomly distributed were neoplastic and non-neoplastic changes.

General observations: consisted of alopecia and thinning of fur in all combination groups and in EE alone groups. Compared to controls, incidence of extended abdomen and body growth retardation was increased in mid and high dose EE groups.
Food and water consumption: EE administration alone or in combination decreased food intake which was increased in mid and high dose DRSP alone groups. Water intake was not affected.
Body weight: Body weight gain was significantly lower in groups treated with combination or EE alone. It was higher for high dose DRSP group. The av. weight gain from week 1 through week 105 for control and 9 treated groups was 183, 154, 148, 137, 158, 128, 99, 180, 184 and 223 g respectively.
Thus expressed as % of the control, the gains for various treated groups were 84,81,75,86,70,54,98,100 and 122% respectively.

Hematology: RBC count (10 /ul) was significantly decreased in groups 3, 4, 6 and 7 with values of 7.6, 7.5, 7.7 and 7.4 compared to 8.0 for the control group. Hb and MCHC were also decreased in these groups. Hct was decreased in group 4, 7, 9 and 10.

Bone marrow: a decrease in nucleated bone marrow cells in group 4 compared to controls was considered treatment-related (0.797 vs 0.891 cells/mg bone marrow). However, an increase in this group for the ratio of immature to mature granulocytopoietic cells and decrease in lymphocyte and plasma cell count were considered as chance occurrences and ~~not~~ biologically significant because of small differences compared to controls.

Necropsy: Macroscopic alterations observed were as follows:

Group#	1	2	3	4	5	6	7	8	9	10
#of animals	110	55	55	55	55	55	55	55	55	55
Survivors	65	34	36	38	35	35	29	40	31	26
Liver foci Liver cysts	4 2	3 1	10 1	21 2	4 3	8 4	11 4	0 2	0 1	0 1
Kidney pelvic dilatation	8	3	0	0	1	6	10	4	0	2
Uterus enlarged thickened hemorrhagic contents	3 5	1 1	1 0	0 0	0 2	5 7	10 15	1 0	0 0	0 0
Ovarian sac dilatation	9	8	3	8	7	18	25	6	3	2

Organ weights: Organs weights are not given. It is not mentioned if organs were weighed or not.
Compilation of necropsy and non-neoplastic histopathological findings and neoplastic histopathological alterations related or suspected to be related to EE and DRSP administered by gavage in combination or alone over 2 years are given in tables 11-16 appended.
The necropsy findings correlated with histologic findings as shown in table on next page.